L5 ANSWER 1 OF 8 BIOTECHDS COPYRIGHT 2004 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2003-21282 BIOTECHDS
TITLE: Microorganism detecting composition comprises **dideoxynucleotide triphosphate**(s) corresponding to one of four **deoxynucleotide triphosphate**, and **thermally stable polymerase** enzyme; **dNTP** and DNA **primer** for DNA sequencing and microorganism detection
AUTHOR: LEUSHNER J; HUI M; DUNN J M; LACROIX J
PATENT ASSIGNEE: LEUSHNER J; HUI M; DUNN J M; LACROIX J
PATENT INFO: US 2003082535 1 May 2003
APPLICATION INFO: US 2001-802110 7 Mar 2001
PRIORITY INFO: US 2001-802110 7 Mar 2001; US 1996-640672 1 May 1996
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-576607 [54]
AN 2003-21282 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - Microorganism detecting composition comprises at least one **dideoxynucleotide triphosphate** which is in a mole ratio to a corresponding **deoxynucleotide triphosphate** of 1:50-1:500, and a **thermally stable polymerase** enzyme that incorporates dideoxynucleotides into an extending nucleic acid polymer at a rate which is not less than 0.4 times the rate of incorporation of deoxynucleotides.

DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for a kit for detection of a target microorganism, which comprises, in packaged combination, a pair of **primers** which bind to the sense and antisense strands, respectively, and flank a selected region within the genome target microorganism; and the inventive microorganism detecting composition.

BIOTECHNOLOGY - Preferred Composition: The mole ratio **dideoxynucleotide triphosphate** to **deoxynucleotide triphosphate** is 1:100-1:300. Preferred Kit: The mole ratio **dideoxynucleotide triphosphate** to its corresponding **deoxynucleotide triphosphate** is 1:100-1:1000, preferably 1:500. At least one of the **primers** is labeled with a fluorescent label. The **primers** are each labeled with a spectroscopically-distinct fluorescent label. The target microorganism is Chlamydia trachomatis, human immunodeficiency virus, or human papilloma virus. The first and second **primers** are oligonucleotides including TCCGGAGCGAGTTACGAAGA; ATTCAATGCCCGGGATTGGT; CCGACCGCGTCTTGAAAACAGATGT; CACCCACATTCCCAGAGAGCT; CGTGCAGCTTTGTGGGAATGT; CTAGATTTCATCTTGTTCAATTGC; AGCATGCGTRTKGGTTACTAYGG; TGACTTTGTTTTCGACCGYGTTTT; CTAAAGTYGCRCATCCACATTCC; CATCCACATTCCCASARAGCTGC; ATGCCCGGGATTGGTTGATC; GGAGACTTTGTTTTCGACCG; CATTCCCACAAAGCTGCGCG; TTCCCACAAAGCTGCGCGAG; CCCACAAAGCTGCGCGAGCG; ACCTTTCGGTTGAGGGAGAGTCTA; GGACCAATTCTTATTCCCAAGCGA; ATCACTCTTTGGCAACGACC; CAGGAGCAGATGATACAGTATTAG; GCMCAGGGWCATAAYAATGG; or CGTCCMAARGGAWACTGATC.

USE - The composition is used for detecting a target microorganism. It is used in a bi-directional DNA sequencing method in several contexts including detection of mutations, particularly mutations of medical significance, in samples derived from a human patient, animal, plant, or microorganism; determination of HLA (human leukocyte antigen) type ancillary to transplant procedures; detection and identification of microorganisms, particularly pathogenic microorganisms, in a sample; and in-situ sequencing reactions to produce sequencing fragments within a histological specimen which are then removed from a selected location on the tissue preparation and loaded onto a gel for sequence analysis.

ADVANTAGE - The invention allows an evaluation to be directly performed on a natural abundance DNA sample. It provides for bi-directional sequencing of DNA which requires combining a complex

DNA-containing sample with only a single reaction mixture, thus reducing risk of error and contamination, and increasing the ease with which the procedure can be automated.

EXAMPLE - Urine samples from patients suspected of carrying a sexually transmitted disease pathogen were prepared for sequence-based diagnosis. One hundred microlitres first void urine were deposited in a sterile microcentrifuge tube. One hundred microlitres Lysis Solution was added to the bacterial pellet and incubated 1 hour at 55 degrees Centigrade, or 18 hours at room temperature. After a final incubation at 95 degrees Centigrade for 10 minutes, 200 microlitres Geneclean II glass milk was added. DNA was eluted in 10 microlitres of double distilled water. The sample natural abundance DNA was treated with a pair of **primers**, i.e. TCCGGAGCGAGTTACGAAGA and ATTCAATGCCCGGGATTGGT, and reagents to identify the sequence of a C. trachomatis gene present in the sample. Three microlitres of the sequencing reaction mixture was placed in each of 4 tubes and covered with 1 drop mineral oil. The tube was heated for 3 minutes at 94 degrees Centigrade and cooled to 85 degrees Centigrade. Termination mixtures were added and the mixture was subjected to thermal cycling for 55 cycles. After the last cycle, the tubes were kept at 70 degrees Centigrade for 2 minutes, and cooled to 4 degrees Centigrade. Reaction products were electrophoretically separated and detected. The base-called sequence was compared to the known C. trachomatis sequence to confirm diagnosis, and results were reported to the patient file.(94 pages)

L5 ANSWER 2 OF 8 BIOTECHDS COPYRIGHT 2004 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2002-17591 BIOTECHDS
TITLE: Preparing a nucleic acid product terminated at a selected base, useful e.g. for diagnostic measurement of the size of telomeric overhangs, from a template that has a break; DNA **primer** and DNA sequencing for disease diagnosis
AUTHOR: MAKAROV V L; LANGMORE J P
PATENT ASSIGNEE: UNIV MICHIGAN
PATENT INFO: US 2002042059 11 Apr 2002
APPLICATION INFO: US 1997-801346 6 Mar 1997
PRIORITY INFO: US 2001-801346 6 Mar 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-478842 [51]
AN 2002-17591 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - Preparation of a nucleic acid product (I), terminated at selected base, by treating a double-stranded (ds) template (II), having at least one break in at least one strand, with a polymerase and terminating composition that contains at least one terminating nucleotide (tnt), corresponding to the selected base.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) sequencing (M1) nucleic acid (NA) by applying the new method to a population of (II) each having at least one random break on at least one strand; (2) mapping (M2) NA by applying the new method to a population of (II) having at least one random break on one strand only; (3) sequencing (M3) through a telomeric repeat region into a subtelomeric region; (4) determining (M4) the length of a single-stranded (ss) overhang of a telomer; and (5) selecting (M5) a (I) terminated at a selected base.

BIOTECHNOLOGY - Preferred Process: (II) is particularly broken in one strand only and is produced by treatment with (i), particularly in NA containing at least one uracil, a combination of two breaking enzymes (BE); (ii) a chemical cleavage composition or (iii) a nuclease, which may introduce specific breaks. To introduce random breaks, ds-DNA containing a randomly positioned exonuclease-resistant nucleotide (especially a phosphorothioate or boranophosphate) is treated with an exonuclease (especially exonuclease III), or with a randomly breaking nuclease,

especially deoxyribonuclease I or CviJI. Alternatively (i) a combination of at least two randomly breaking enzymes (rBE), especially frequent cutters; (ii) a randomly breaking chemical cleavage complex; (iii) gamma-radiation; (iv) mechanical forces or (v) freeze-thaw cycles are used. The break is specifically a nick or gap and comprises a 3'-hydroxy. (I) is then detected, especially as part of a sequencing or mapping procedure. Optionally it is isolated, by electrophoresis, mass spectrometry or chromatography. Where the break is random, at least one specific nucleotide (snt) is introduced before termination, especially (II) is treated with 4 extension nucleotides (nt) and one tnt to create a population of (I) terminated at the selected base, and at least one of the extending nt is degradable. This method can be used to identify a selected dinucleotide, especially by blocking (II) with three dideoxynucleotide triphosphates (**ddNTP**) that are not the complement of the first base, removing the **ddNTP**, then treatment with an extension and terminating composition containing a **deoxynucleotide triphosphate** (**dNTP**) containing the complement of the first base and a labeled **ddNTP** containing the complement of the second base so that the (I) produced includes a dinucleotide sequence complementary to the two bases. Optionally a series of blocking/extension reactions is performed before termination. when two specified bases are introduced before termination, the method is used to identify a selected trinucleotide sequence. A population of (I) terminated at 4 selected bases may be produced by contacting (II) with 4 terminating bases. The process is then used for sequencing by detecting the population of (I) produced. The template may be treated with an extending nt and each terminating base carries a different fluorescent label. In M3, an NA that comprises, in order, terminal ss telomeric overhang, ds telomeric region and ds subtelomeric region is treated with a **primer** that hybridizes to the overhang, polymerase extension nt and at least one labeled tnt. M4 comprises reacting the overhang with excess of **primer** and quantifying the bound **primer**. Optionally the hybridized **primer** is treated with a ligation composition and the length of the ligated **primer** is measured. In M5, (II) is treated with a composition comprising polymerase and either terminating composition, corresponding to a selected base or to an extension composition to produce a fully extended product only for a template that terminates at the selected base. This process can be used to determine the positions of selected di- or tri-nucleotide sequences. Preferred Materials: (II) is circular or linear, optionally formed by cleaving, or amplification from, a precursor. Suitable specific chemical cleavage compositions are triple-helix formers and random cleaving complexes are able to generate hydroxy radicals, e.g. a mixture of chelating agent, metal ion, reducing agent and peroxide. Preferred Composition: The termination composition contains terminating (di)**deoxynucleotide triphosphate** corresponding to the selected base, and this may be labeled or include an isolation tag, and similar groups may be present on (II). Suitable labels are radioactive, enzymatic or fluorescent and the isolation tag is particularly biotin.

USE - The method is useful for sequencing, especially through a telomeric repeat region, or mapping of nucleic acid, e.g. to determine the location of specific di- or tri-nucleotide segments; also to determine the length of telomer overhangs (claimed), potentially useful for diagnosing chromosomal instabilities caused by telomerase, nuclease, recombination or other effects, important in cancer and age-related disorders.

ADVANTAGE - The method provides accurate sequencing (because extension reactions are very short) and produces more useful data from large templates, overcoming problems inherent in single-stranded sequencing techniques.

EXAMPLE - No suitable example is given.(103 pages)

L5 ANSWER 3 OF 8 BIOTECHDS COPYRIGHT 2004 THE THOMSON CORP. on STN

ACCESSION NUMBER: 2001-10134 BIOTECHDS
TITLE: Composition for detecting microorganisms, comprising deoxynucleotide triphosphates, **dideoxynucleotide triphosphate**, and **thermostable polymerase** to incorporate **dideoxynucleotide triphosphate** into extending polymer; DNA **primer** for Chlamydia trachomatis, HIV virus and human papilloma virus detection or serotyping and infection diagnosis
AUTHOR: Leushner J; Hui M; Dunn J M; LaCroix J M
PATENT ASSIGNEE: Visible-Genetics
LOCATION: Toronto, Ontario, Canada.
PATENT INFO: US 6214555 10 Apr 2001
APPLICATION INFO: US 1999-311260 13 May 1999
PRIORITY INFO: US 1999-311260 13 May 1999
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2001-289718 [30]
AN 2001-10134 BIOTECHDS
AB A composition (I) containing a mixture of four deoxynucleotide triphosphates (dNTPs) and at least one **dideoxynucleotide triphosphate** (**ddNTP**) corresponding to one of the four dNTPs, at a molar ratio of 1:50 to 1:500, and a **thermally stable polymerase** enzyme (II) which incorporates **ddNTP** into an extending DNA polymer at a rate not less than 0.4-fold the rate of incorporation of dNTPs, is claimed. Also claimed is a kit for detecting a target microorganism containing, in packaged combination, a pair of DNA **primers** which bind to the sense and antisense strands, respectively, and flank a secreted region within the genome target microorganism, a mixture of four dNTPs and at least one **ddNTP**, at a molar ration of 1:50 to 1:1,000, and (II). (I) and the kit are useful for detecting a target microorganism e.g. Chlamydia trachomatis, HIV virus and human papilloma virus. The method follows a simple test format which is generally applicable to the detection of microorganisms, including infectious disease-causing microorganisms, and particularly for a simple test which provides an indication of the specific nature, e.g. the serotype or the organism. (62pp)

L5 ANSWER 4 OF 8 BIOTECHDS COPYRIGHT 2004 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1998-01940 BIOTECHDS
TITLE: Determination of DNA base sequence and a sequence reagent; DNA sequencing by polymerase chain reaction and chain termination, with fluorescence label
PATENT ASSIGNEE: Shimadzu
LOCATION: Japan.
PATENT INFO: JP 09271400 21 Oct 1997
APPLICATION INFO: JP 1996-108482 3 Apr 1996
PRIORITY INFO: JP 1996-108482 3 Apr 1996
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
OTHER SOURCE: WPI: 1998-003031 [01]
AN 1998-01940 BIOTECHDS
AB A new method for DNA sequencing in which a DNA **primer** is annealed to a template DNA, involves: using a **Taq** DNA-**polymerase** (EC-2.7.7.7) in the presence of each **ddNTP** (corresponding to 4 dNTPs) and 4 bases to add **dNTP** at the 3' terminal end of the **primer** to extend the chain; stopping the reaction by incorporating **ddNTP**; repeating the reaction to effect a terminal label polymerase chain reaction (PCR); synthesizing DNA of different chain lengths by PCR; and analyzing the sequence. The labeled **ddNTP** is preferably e.g. ddATP, ddGTP, ddUTP, ddTTP, which are labeled using a fluorescein pigment. Also claimed is a sequence reagent used for the above method, containing an enzyme for amino acid modification of **Taq** DNA-**polymerase**. The

method may be used to give reliable analytical data of base sequence. In an example, fluorescein-12(F12)-ddATP NEL-402 was used as the fluorescein-labeled (FL) ddATP, F12-ddGTP NEL-403 was used as the FL ddGTP, F12-ddCTP NEL-400 was used as the FL ddCTP, and F12-ddUTP NEL-401 was used as the FL ddUTP. (9pp)

L5 ANSWER 5 OF 8 BIOTECHDS COPYRIGHT 2004 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1990-08044 BIOTECHDS
TITLE: Nucleotide sequence determination by chain termination; DNA sequencing method by dideoxy chain termination using Thermus aquaticus **Taq** DNA-**polymerase**; asymmetric **polymerase** chain reaction
PATENT ASSIGNEE: Cetus
PATENT INFO: WO 9003442 5 Apr 1990
APPLICATION INFO: WO 1989-US4093 19 Sep 1989
PRIORITY INFO: US 1988-249367 23 Sep 1988
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 1990-132278 [17]
AN 1990-08044 BIOTECHDS
AB A method for DNA sequencing by dideoxynucleotide-5'-triphosphate (**ddNTP**) chain termination is claimed, where an oligonucleotide DNA **primer** is extended in a template-dependent manner in the presence of Thermus aquaticus **Taq** DNA-**polymerase** (EC-2.7.7.7), 4 deoxyribonucleotide-5'-triphosphates (dNTPs: dATP, dCTP, dGTP/c7dGTP/dITP and TTP) and a **ddNTP**. The **primer** and 1 of the dNTPs are labeled. The DNA fragment may be produced by an asymmetric polymerase chain reaction. The reaction mixture contains no KCl, and the DNA-polymerase is present at up to 2.5-fold molar excess over the DNA fragment. The extension reaction is carried out at a low temperature in the presence of 3 unlabeled dNTPs (at 1.0 uM each) and 1 labeled **dNTP** at less than 1 uM (preferably 0.5 uM) and then at higher temperature levels in higher concentrations of the unlabeled dNTPs (5-30 uM, preferably 10 uM). The dATP:ddATP ratio is 1:32, the dCTP:ddCTP ratio is 1:16, the dGTP:ddGTP ratio is 1:6, and the TTP:ddTTP ratio is 1:48. This method allows DNA-polymerase to read through hairpin structures, and destabilization with c7dGTP allows resolution of sequencing products. (30pp)

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=> s l1 and kit
L2          71 L1 AND KIT

=> s (primer# and (dNTP or deoxynucleotide triphosphate)
UNMATCHED LEFT PARENTHESIS '(PRIMER#'
The number of right parentheses in a query must be equal to the
number of left parentheses.

=> s (primer# and (dNTP or deoxynucleotide triphosphate))
L3        2743 (PRIMER# AND (DNTP OR DEOXYNUCLEOTIDE TRIPHOSPHATE))

=> s l3 and (ddNTP or dideoxynucleotide triphosphate)
L4         115 L3 AND (DDNTP OR DIDEOXYNUCLEOTIDE TRIPHOSPHATE)

=> s l4 and (thermostable polymerase or thermal### stable polymerase or Taq (3a)
polymerase)
L5           8 L4 AND (THERMOSTABLE POLYMERASE OR THERMAL### STABLE POLYMERASE
                OR TAQ (3A) POLYMERASE)

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L5    ANSWER 1 OF 8  BIOTECHDS COPYRIGHT 2004 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2003-21282  BIOTECHDS
TITLE:            Microorganism detecting composition comprises
                  dideoxynucleotide triphosphate(s)
                  corresponding to one of four deoxynucleotide
                  triphosphate, and thermally stable
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                       dNTP and DNA primer for DNA sequencing
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AUTHOR:           LEUSHNER J; HUI M; DUNN J M; LACROIX J
PATENT ASSIGNEE:  LEUSHNER J; HUI M; DUNN J M; LACROIX J
PATENT INFO:      US 2003082535 1 May 2003
APPLICATION INFO: US 2001-802110 7 Mar 2001
PRIORITY INFO:    US 2001-802110 7 Mar 2001; US 1996-640672 1 May 1996
DOCUMENT TYPE:    Patent
LANGUAGE:         English
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           DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for a
      kit for detection of a target microorganism, which comprises, in packaged
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      antisense strands, respectively, and flank a selected region within the
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           BIOTECHNOLOGY - Preferred Composition: The mole ratio
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      Kit: The mole ratio dideoxynucleotide triphosphate to
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      microorganism is Chlamydia trachomatis, human immunodeficiency virus, or
      human papilloma virus. The first and second primers are
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oligonucleotides including TCCGGAGCGAGTTACGAAGA; ATTCAATGCCCGGGATTGGT; CCGACCGCGTCTTGAAAACAGATGT; CACCCACATTCCCAGAGAGCT; CGTGCAGCTTTGTGGGAATGT; CTAGATTTCATCTTGTTCAATTGC; AGCATGCGTRTKGGTTACTAYGG; TGACTTTGTTTTCGACCGYGTTTT; CTAAAGTYGCRCATCCACATTCC; CATCCACATTCCCASARAGCTGC; ATGCCCGGGATTGGTTGATC; GGAGACTTTGTTTTCGACCG; CATTCCCACAAAGCTGCGCG; TTCCCACAAAGCTGCGCGAG; CCCACAAAGCTGCGCGAGCG; ACCTTTCGGTTGAGGGAGAGTCTA; GGACCAATTCTTATTCCCAAGCGA; ATCACTCTTTGGCAACGACC; CAGGAGCAGATGATACAGTATTAG; GCMCAGGGWCATAAYAATGG; or CGTCCMAARGGAWACTGATC.

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ADVANTAGE - The invention allows an evaluation to be directly performed on a natural abundance DNA sample. It provides for bi-directional sequencing of DNA which requires combining a complex DNA-containing sample with only a single reaction mixture, thus reducing risk of error and contamination, and increasing the ease with which the procedure can be automated.

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ACCESSION NUMBER: 2002-17591 BIOTECHDS
TITLE: Preparing a nucleic acid product terminated at a selected base, useful e.g. for diagnostic measurement of the size of telomeric overhangs, from a template that has a break; DNA **primer** and DNA sequencing for disease diagnosis
AUTHOR: MAKAROV V L; LANGMORE J P
PATENT ASSIGNEE: UNIV MICHIGAN
PATENT INFO: US 2002042059 11 Apr 2002
APPLICATION INFO: US 1997-801346 6 Mar 1997
PRIORITY INFO: US 2001-801346 6 Mar 2001
DOCUMENT TYPE: Patent
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AB DERWENT ABSTRACT:
NOVELTY - Preparation of a nucleic acid product (I), terminated at

selected base, by treating a double-stranded (ds) template (II), having at least one break in at least one strand, with a polymerase and terminating composition that contains at least one terminating nucleotide (tnt), corresponding to the selected base.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) sequencing (M1) nucleic acid (NA) by applying the new method to a population of (II) each having at least one random break on at least one strand; (2) mapping (M2) NA by applying the new method to a population of (II) having at least one random break on one strand only; (3) sequencing (M3) through a telomeric repeat region into a subtelomeric region; (4) determining (M4) the length of a single-stranded (ss) overhang of a telomer; and (5) selecting (M5) a (I) terminated at a selected base.

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hydroxy radicals, e.g. a mixture of chelating agent, metal ion, reducing agent and peroxide. Preferred Composition: The termination composition contains terminating (di)**deoxynucleotide triphosphate** corresponding to the selected base, and this may be labeled or include an isolation tag, and similar groups may be present on (II). Suitable labels are radioactive, enzymatic or fluorescent and the isolation tag is particularly biotin.

USE - The method is useful for sequencing, especially through a telomeric repeat region, or mapping of nucleic acid, e.g. to determine the location of specific di- or tri-nucleotide segments; also to determine the length of telomer overhangs (claimed), potentially useful for diagnosing chromosomal instabilities caused by telomerase, nuclease, recombination or other effects, important in cancer and age-related disorders.

ADVANTAGE - The method provides accurate sequencing (because extension reactions are very short) and produces more useful data from large templates, overcoming problems inherent in single-stranded sequencing techniques.

EXAMPLE - No suitable example is given.(103 pages)

L5 ANSWER 3 OF 8 BIOTECHDS COPYRIGHT 2004 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2001-10134 BIOTECHDS
TITLE: Composition for detecting microorganisms, comprising deoxynucleotide triphosphates, **dideoxynucleotide triphosphate**, and **thermostable polymerase** to incorporate **dideoxynucleotide triphosphate** into extending polymer; DNA **primer** for Chlamydia trachomatis, HIV virus and human papilloma virus detection or serotyping and infection diagnosis
AUTHOR: Leushner J; Hui M; Dunn J M; LaCroix J M
PATENT ASSIGNEE: Visible-Genetics
LOCATION: Toronto, Ontario, Canada.
PATENT INFO: US 6214555 10 Apr 2001
APPLICATION INFO: US 1999-311260 13 May 1999
PRIORITY INFO: US 1999-311260 13 May 1999
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2001-289718 [30]
AN 2001-10134 BIOTECHDS
AB A composition (I) containing a mixture of four deoxynucleotide triphosphates (dNTPs) and at least one **dideoxynucleotide triphosphate** (**ddNTP**) corresponding to one of the four dNTPs, at a molar ratio of 1:50 to 1:500, and a **thermally stable polymerase** enzyme (II) which incorporates **ddNTP** into an extending DNA polymer at a rate not less than 0.4-fold the rate of incorporation of dNTPs, is claimed. Also claimed is a kit for detecting a target microorganism containing, in packaged combination, a pair of DNA **primers** which bind to the sense and antisense strands, respectively, and flank a secreted region within the genome target microorganism, a mixture of four dNTPs and at least one **ddNTP**, at a molar ration of 1:50 to 1:1,000, and (II). (I) and the kit are useful for detecting a target microorganism e.g. Chlamydia trachomatis, HIV virus and human papilloma virus. The method follows a simple test format which is generally applicable to the detection of microorganisms, including infectious disease-causing microorganisms, and particularly for a simple test which provides an indication of the specific nature, e.g. the serotype or the organism. (62pp)

L5 ANSWER 4 OF 8 BIOTECHDS COPYRIGHT 2004 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1998-01940 BIOTECHDS
TITLE: Determination of DNA base sequence and a sequence reagent; DNA sequencing by polymerase chain reaction and chain termination, with fluorescence label

PATENT ASSIGNEE: Shimadzu
LOCATION: Japan.
PATENT INFO: JP 09271400 21 Oct 1997
APPLICATION INFO: JP 1996-108482 3 Apr 1996
PRIORITY INFO: JP 1996-108482 3 Apr 1996
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
OTHER SOURCE: WPI: 1998-003031 [01]
AN 1998-01940 BIOTECHDS
AB A new method for DNA sequencing in which a DNA **primer** is annealed to a template DNA, involves: using a **Taq** DNA-**polymerase** (EC-2.7.7.7) in the presence of each **ddNTP** (corresponding to 4 dNTPs) and 4 bases to add **dNTP** at the 3' terminal end of the **primer** to extend the chain; stopping the reaction by incorporating **ddNTP**; repeating the reaction to effect a terminal label polymerase chain reaction (PCR); synthesizing DNA of different chain lengths by PCR; and analyzing the sequence. The labeled **ddNTP** is preferably e.g. ddATP, ddGTP, ddUTP, ddTTP, which are labeled using a fluorescein pigment. Also claimed is a sequence reagent used for the above method, containing an enzyme for amino acid modification of **Taq** DNA-**polymerase**. The method may be used to give reliable analytical data of base sequence. In an example, fluorescein-12(F12)-ddATP NEL-402 was used as the fluorescein-labeled (FL) ddATP, F12-ddGTP NEL-403 was used as the FL ddGTP, F12-ddCTP NEL-400 was used as the FL ddCTP, and F12-ddUTP NEL-401 was used as the FL ddUTP. (9pp)

L5 ANSWER 5 OF 8 BIOTECHDS COPYRIGHT 2004 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1990-08044 BIOTECHDS
TITLE: Nucleotide sequence determination by chain termination; DNA sequencing method by dideoxy chain termination using Thermus aquaticus **Taq** DNA-**polymerase**; asymmetric **polymerase** chain reaction
PATENT ASSIGNEE: Cetus
PATENT INFO: WO 9003442 5 Apr 1990
APPLICATION INFO: WO 1989-US4093 19 Sep 1989
PRIORITY INFO: US 1988-249367 23 Sep 1988
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 1990-132278 [17]
AN 1990-08044 BIOTECHDS
AB A method for DNA sequencing by dideoxynucleotide-5'-triphosphate (**ddNTP**) chain termination is claimed, where an oligonucleotide DNA **primer** is extended in a template-dependent manner in the presence of Thermus aquaticus **Taq** DNA-**polymerase** (EC-2.7.7.7), 4 deoxyribonucleotide-5'-triphosphates (dNTPs: dATP, dCTP, dGTP/c7dGTP/dITP and TTP) and a **ddNTP**. The **primer** and 1 of the dNTPs are labeled. The DNA fragment may be produced by an asymmetric polymerase chain reaction. The reaction mixture contains no KCl, and the DNA-polymerase is present at up to 2.5-fold molar excess over the DNA fragment. The extension reaction is carried out at a low temperature in the presence of 3 unlabeled dNTPs (at 1.0 uM each) and 1 labeled **dNTP** at less than 1 uM (preferably 0.5 uM) and then at higher temperature levels in higher concentrations of the unlabeled dNTPs (5-30 uM, preferably 10 uM). The dATP:ddATP ratio is 1:32, the dCTP:ddCTP ratio is 1:16, the dGTP:ddGTP ratio is 1:6, and the TTP:ddTTP ratio is 1:48. This method allows DNA-polymerase to read through hairpin structures, and destabilization with c7dGTP allows resolution of sequencing products. (30pp)

=> d his

(FILE 'HOME' ENTERED AT 16:10:45 ON 16 DEC 2004)

FILE 'MEDLINE, BIOTECHDS, EMBASE, BIOSIS, SCISEARCH, CANCERLIT, CAPLUS'
ENTERED AT 16:11:25 ON 16 DEC 2004
L1 28431 S LEUSHNER J?/AU OR HUI ?/AU OR DUNN J?/AU
L2 71 S L1 AND KIT
L3 2743 S (PRIMER# AND (DNTP OR DEOXYNUCLEOTIDE TRIPHOSPHATE))
L4 115 S L3 AND (DDNTP OR DIDEOXYNUCLEOTIDE TRIPHOSPHATE)
L5 8 S L4 AND (THERMOSTABLE POLYMERASE OR THERMAL### STABLE POLYMER

=> s l4 and kit
L6 16 L4 AND KIT

=> dup rem l6
PROCESSING COMPLETED FOR L6
L7 15 DUP REM L6 (1 DUPLICATE REMOVED)

=> d ibib abs l7 1-15

L7 ANSWER 1 OF 15 BIOTECHDS COPYRIGHT 2004 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2004-23661 BIOTECHDS
TITLE: Detecting mutations in a nucleic acid sequence, useful for analyzing multiple mutations in the nucleic acid sequence, comprises amplifying nucleic acid fragment by LA-PCR in order to obtain a target DNA;
LA-polymerase chain reaction for target DNA mutation identification and pharmacogenetics
AUTHOR: KIHLGREN A; MOLANDER C; ROSEN B
PATENT ASSIGNEE: DYNAMIC CODE AB
PATENT INFO: WO 2004087964 14 Oct 2004
APPLICATION INFO: WO 2004-EP521 2 Apr 2004
PRIORITY INFO: SE 2003-965 2 Apr 2003; SE 2003-965 2 Apr 2003
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2004-737736 [72]
AN 2004-23661 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - Detecting mutations in a nucleic acid sequence, which consists in determining at least part of that nucleic acid sequence, comprises amplifying at least one nucleic acid fragment from an isolated nucleic acid specimen by LA-PCR in order to obtain a target DNA.

DETAILED DESCRIPTION - Detecting mutations in a nucleic acid sequence, which consists in determining at least part of that nucleic acid sequence, comprises: (a) amplifying at least one nucleic acid fragment from an isolated nucleic acid specimen by LA-PCR in order to obtain a target DNA; (b) designing oligonucleotide **primer** which are at least partially complementary to the target DNA, those with largely different sequences having different numbers of nucleotides; (c) hybridizing the oligonucleotide **primers** with the target DNA; (d) extending the oligonucleotide **primers** with detectably labeled, terminating nucleotides; (e) separating the extended oligonucleotide **primers** according to size; and (f) identifying extended nucleotides by labeling of the terminating nucleotides. An INDEPENDENT CLAIM is also included for a **kit**, for performing the method above, comprising oligonucleotide **primer** array for performing steps (a) and (b) and detectably labeled terminating nucleotides.

BIOTECHNOLOGY - Preferred Method: The nucleic acid specimen derives from blood, buccal specimens or hair roots. The isolated nucleic acid specimen comprises mRNA or DNA. The target DNA obtained by LA-PCR comprises fragments of 0.5-100, 5-100, or 10-100 kb. At least 3 or 5 oligonucleotide **primers** are designed in step (b) and are then used to perform steps (c) to (f) with the same reaction mix. The target DNA derives from at least one gene. The target DNA derives from at least

one pharmaco-gene. The method further comprises determining the number of gene copies in a DNA specimen. The method comprises using RT-PCR to determine the number of gene copies in the DNA specimen and the number of copies of each gene being determined relative to a control, which is a gene that has not been found to be duplicated or replicated in a cell. The detectably labeled, terminating nucleotides are deoxynucleoside triphosphate (**dNTP**) or dideoxynucleoside triphosphate (**ddNTP**). The detectably labeled, terminating nucleotides are synthetically modified. The detectably labeled, terminating nucleotides are labeled with fluorescent dyes, chemiluminescent reagents or radioactive groups. Preferred **Kit**: The **kit** also comprises a **primer** and probe array for determining the number of copies in the DNA specimen and the number of copies of each gene being determined relative to a control, which is a gene that has not been found to be duplicated or replicated in a cell.

USE - The method and **kit** are useful for detecting and analyzing mutations in a nucleic acid sequence. The method is also useful for analyzing multiple mutations in the nucleic acid sequence. The **kit** can also be used for determining a pharmaceutical or therapeutic substance for an individual and a suitable dosage interval for this substance.

EXAMPLE - DNA was isolated from cells. The number of gene copies was determined. Twenty-five microlitres universal PCR mix, 2.5 microlitres **primer** and probe mix, 2.5 microlitres **primer** and probe mix for CYP3A5 and 19 microlitres ddH2O were mixed and 1 microlitres DNA (50 pg - 50 ng) was then added. A PCR amplification was performed. Results were analyzed. The pharmaco-gene CYP3A5 occurred in the same number of copies as the internal control, which means that no allele has been removed or duplicated. LA-PCR was performed. The following were mixed: 6 microlitres XL buffer, 4 microlitres deoxynucleoside triphosphates (dNTPs), 1 microlitres each of the **primers**, 1.6 microlitres Mg(OAc)2 and 6.4 microlitres ddH2O, which gave a total volume of 20 microlitres. A wax pellet was then added to the specimen, made to melt, and then solidified at room temperature. The following were mixed on top of the wax layer: 9 microlitres 3.3 XL buffer, 0.5 microlitres rTth DNA polymerase, 1 microlitres DNA and 19.5 microlitres ddH2O, which gave a total volume of 30 microlitres. PCR was performed. The result was a PCR product of 36.9 kb. Mutations were then analyzed. The following were mixed in a tube on ice: 5 microlitres PCR mix from **kit**, 3 microlitres PCR product, 1 microlitres **primer** mix and 1 microlitres ddH2O. The tube was placed in a thermocycler. The specimens were incubated with 1 U alkaline phosphatase for an hour at 37degreesC and phosphatase was inactivated for 15 minutes at 75degreesC. The PCR products were analyzed. Size of the **primers** was determined relative to internal size standard. The nucleotides were used to extend the **primers**. There were 2 **primers** extended with 2 different nucleotides. Results showed that the individual carries 1 CYP3A5asterisk1 allele, which is a wild type allele and 1 CYP3A5asterisk3A allele, which is an allele which gives an inactive enzyme. The individual is so-called intermediate metabolizer.(17 pages)

L7 ANSWER 2 OF 15 BIOTECHDS COPYRIGHT 2004 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2004-19953 BIOTECHDS
TITLE: Analyzing multiple targets in polynucleotide, by providing multiple **primers** with target nucleic acids, digesting nucleic acid products with cognate restriction enzymes, amplifying digested products, and detecting amplified products;
target polynucleotide analysis and **primer** extension for use in mutation genotyping
AUTHOR: FU G
PATENT ASSIGNEE: FU G
PATENT INFO: US 2004146866 29 Jul 2004
APPLICATION INFO: US 2003-349780 24 Jan 2003

PRIORITY INFO: US 2003-349780 24 Jan 2003; US 2003-349780 24 Jan 2003
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2004-552653 [53]
AN 2004-19953 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - Analyzing multiple targets in polynucleotide, involves providing a set of multiple **primers** with target nucleic acids in reactions of **primer** extension or amplification, where the reaction produce nucleic acid products, digesting nucleic acid products of the reactions on the restriction sites with cognate restriction enzymes, joining digested products, amplifying the joined products, and detecting the amplified products.

DETAILED DESCRIPTION - Analyzing (M1) multiple targets in polynucleotide, involves providing a set or sets of multiple **primers** with target nucleic acids in separate reactions of **primer** extension or amplification, where the reaction produce nucleic acid products in that each nucleic acid fragments comprise at least one restriction site, digesting nucleic acid products of the separate reactions on the restriction sites with cognate restriction enzymes, joining digested products derived from the separate reactions together, where randomly joining nucleic acid fragments from the separated reactions are created, amplifying the joined products, and detecting the amplified products. INDEPENDENT CLAIMS are also included for: (1) an oligonucleotide **primer** (I) for detecting target nucleic acid sequence, comprises 3' complementary portion and 5' non-complementary portion, where the 5' non-complementary portion comprises a restriction enzyme site, where the restriction site acts as detection marker in the process of detecting target nucleic acid sequence, where the detection signal generated from enzymatic manipulation on restriction site of reaction product is indicative of the presence of target nucleic acid sequence; and (2) a **kit** for use in analysis and detection of multiple targets in a polynucleotide, comprising a set or sets of multiple **primers**, universal **primers**, restriction enzymes, DNA ligase, DNA polymerase, **ddNTP**, buffers for all enzymes, and dNTPs.

BIOTECHNOLOGY - Preferred Method: In (M1), the **primers** are oligonucleotides comprising 3' complementary portion or 3' complementary portion and 5' non-complementary portion. The set or sets of multiple **primer** comprise mixtures of target specific **primers**, where the **primer** pairs of forward **primers** are reverse **primers** specific for each target are included. The set or sets of multiple **primer** comprise mixtures of target specific reverse **primers** and universal **primer**, where the universal **primers** comprise sequence identical or homologous to non-complementary portion of target specific forward **primers**. The **primers** or subset of **primers** comprise capture moiety. The capture moiety is biotin. The **primer** extension is first strand cDNA synthesis from target RNA in the presence of set of target specific **primers**, random **primers** or oligo dT **primers**. The **primer** extension is second strand cDNA synthesis in the presence of a set of target specific **primers** or random **primers**. The amplification is PCR carried out at least once for 1-30 cycles, preferably 3-15 cycles. (M1) further involves purifying and isolating the nucleic acid products before and/or after digesting on the restriction sites with cognate restriction enzymes. The purification and isolation steps comprise immobilizing the nucleic acid product on a solid support. The solid support is streptavidin coated beads. The joining is by ligation using a DNA ligase. The amplifying is performed using a set or sets of multiple **primers** or using universal **primers** having sequences identical or homologous to non-complementary portions of target specific **primers**. The universal **primers** comprise fluorescence dye labels. The restriction sites are located on target sequences or on **primer**

sequences, where the locations of restriction sites are chosen such that the amplification products digested on the restriction site are distinguishable by their sizes and/or labels. The restriction sites are the same restriction sites for all nucleic acid fragments generated in the reaction. The restriction sites are different and specific for subset of targets. The detecting is electrophoresis. The multiple targets comprise single nucleotide polymorphisms (SNPs) or mutations. The set or sets of multiple **primer** comprise mixture of reverse **primers** and allele-specific forward **primers**, where two allele-specific forward **primers** and one common reverse **primers** for each target are included. The allele-specific forward **primers** comprise 3' ends which are complementary to either allele at mutation or polymorphism sites. The two allele-specific forward **primers** comprise allele-specific restriction site that is different and specific for each allele and is located 5' of the complementary portion of allele-specific forward **primers**. The two allele-specific forward **primers** comprise the same restriction sites having different locations. The allele-specific forward **primers** comprise first and second restriction sites in non-complementary portion of each **primer**, where the first restriction sites on all forward **primers** in a set of multiple **primers** are the same restriction site and are located 5' of second restriction sites, where the second restriction sites are allele-specific restriction sites which are different and specific for each of two allele-specific forward **primers** and are located 5' of the complementary portion of allele-specific forward **primers** . The second restriction sites produce 5' protruding ends after digestion. The step of detecting amplified products involves purifying amplified products, digesting amplified products, extending with a DNA polymerase in the presence of fluorescence dye labeled terminators, and putting extended DNA product into a electrophoresis system. The step of purifying amplified products comprises eliminating **dNTP** and **primers**. The elimination step involves incubating with shrimp alkaline phosphatase and exonuclease I. The digesting involves digesting amplified products on the first or second restrictions sites. The terminators are **ddNTP**. The electrophoresis system is a gel, capillary electrophoresis system, or DNA sequencer. Preferred **Primer**: In (I), the restriction site is allele-specific, gene-specific or SNP-specific.

USE - (M1) is useful for analyzing multiple targets in a polynucleotide (claimed). (M1) is useful for genotyping mutations, preferably single nucleotide polymorphisms (SNPs), and for analyzing differential gene expression profiles, genomic methylation patterns and any specific nucleic acids from any source.

ADVANTAGE - (M1) enables to analyze multiple targets quantitatively.

EXAMPLE - Two universal **primers** M13F and M13R were designed, M13F was tagged with biotin. Two allele-specific forward **primers** were designed for each target. The forward **primers** comprised 3' terminal complementary portion and 5' terminal non-complementary portions which comprise a common first restriction site (EcoRI) and allele-specific second restriction sites (DpnII and MspI) that are specific for each allele. Two reverse **primers** were designed for each target, the nested reverse **primers** comprised complementary portion and non-complementary portion. Human genomic DNA samples were prepared by standard extraction from blood cells. **Primers** were diluted. Singleplex PCR were performed on each pair of **primer** which showed that the **primer** pairs for single nucleotide polymorphism (SNP) 7 and 8 failed. The above product was purified, and bound with biotinylated DNA onto magnetic beads. Restriction digestion, precipitation, and ligation steps were carried out. The product was amplified. All working **primers** pairs of 8 SNPs gave clear either heterozygous or homozygous patterns. The EcoRI lane revealed all 8 fragments, the MspI lane revealed fragments corresponding alleles that were produced with

MspI tagged allele-specific **primers**, the DpnII lane revealed fragments corresponding other alleles that were produced with DpnII I tagged allele-specific **primers**. (65 pages)

L7 ANSWER 3 OF 15 BIOTECHDS COPYRIGHT 2004 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2004-21806 BIOTECHDS
TITLE: In vitro screening of compounds that inhibit reverse transcription, useful for treatment and prevention of human immune deficiency virus infection, from their effect on a **primer**-matrix complex;
drug screening using RNA **primer**-RNA matrix complex for use in HIV virus-1 prevention and therapy
AUTHOR: BRULE F; ISEL C; MARQUET R; RIGOURD M
PATENT ASSIGNEE: CNRS CENT NAT RECH SCI
PATENT INFO: FR 2851577 27 Aug 2004
APPLICATION INFO: FR 2003-50029 20 Feb 2003
PRIORITY INFO: FR 2003-50029 20 Feb 2003; FR 2003-50029 20 Feb 2003
DOCUMENT TYPE: Patent
LANGUAGE: French
OTHER SOURCE: WPI: 2004-618220 [60]
AN 2004-21806 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - In vitro method of screening for compounds (A) that inhibit the initiation of reverse transcription of RNA of HIV-1.

DETAILED DESCRIPTION - In vitro method of screening for compounds (A) that inhibit the initiation of reverse transcription of RNA of HIV-1 comprises: (a) incubating together a complex (C) of **primer** RNA (P) and matrix RNA (M); HIV-1 reverse transcriptase (RT); mixture of (di)deoxynucleotidfe triphosphates ((d)**dNTP**) and test compound; (b) measuring the activity of initiation of reverse transcription; and (c) comparing this activity with that in a control sample that does not contain test compound. (P), which is either an in vitro transcript from the appropriate nucleic acid or a (semi-)synthetic product, comprises, in the 5' to 3' direction, an intramolecular pairing sequence (109); single-stranded sequence (111); sequence (112) that forms a stem-loop; single-stranded region (113); intermolecular pairing sequence (101) ('anticodon'); intermolecular pairing sequences (103) and (105); intramolecular pairing sequence (110); single-stranded sequence (114) and intermolecular pairing sequence (107), functioning as **primer**. (M) comprises, in the 5' to 3' direction, intramolecular pairing sequences (115) and (116); intermolecular pairing sequences (106); sequence (117) that forms an intramolecular stem-loop; intramolecular pairing sequence (104); intramolecular pairing sequence (102) ('codon'); intramolecular pairing sequence (118); single-stranded sequence (119); intermolecular pairing sequence (108); sequence (120) forming an intramolecular stem-loop; single-stranded sequence (121) and intramolecular pairing sequence (122). When paired, (101) and (102) form helix 6C; (107) and (108) form helix 7F; (109) and (110) form helix A; (115) and (122) form helix 1; (116) and (118) form helix 2; while (112), (117) and (120) form stem-loops B, 4 and 8. INDEPENDENT CLAIMS are also included for the following: (1) (C) as a new product; and (2) **kit** for the process that contains (C).

BIOTECHNOLOGY - Preferred Process: This includes selection of compounds that inhibit initiation of reverse transcription and calculation of their inhibitory activity. The mixture of (d)**dNTP** comprises 3 **dNTP** and one **ddNTP**, complementary to the nucleotide at position -6 with respect to the 3'-end of the binding site for **primer** (108) on the matrix. At least one (d)**dNTP** is labeled, particularly by tritium, carbon-14 or phosphorus-32 or -33. (C) is immobilized on a support and at least one RNA in (C) includes a ligand, particularly biotin, that can bind specifically to a molecular receptor on the surface of a support. The process is performed in a reaction vessel, the surface of which is the support for (C), and reverse transcription activity is measured from the amount of radioactivity

present in the polynucleotide synthesized by elongation of hybridized (P). Preferred Materials: The **primer** is a 75 nucleotide (nt) sequence (1) and the matrix a 108 nt sequence (2); or the **primer** is a 76 nt sequence (3) and the matrix a 110 nt sequence (4), all reproduced.

ACTIVITY - Anti-HIV. No details of tests for anti-HIV activity are given.

MECHANISM OF ACTION - Inhibiting reverse transcription of HIV-1 RNA.

USE - The method is used to identify agents for preventative and curative treatment of HIV-1 infections.

ADVANTAGE - The method identifies compounds that are active in the early stages of HIV-1 infection. Complex (C) are easily prepared, in large amounts, by simple in vitro transcription, eliminating the need for long, complex and costly purification from natural sources, and without any post-transcriptional modification of nucleotides. (59 pages)

L7 ANSWER 4 OF 15 BIOTECHDS COPYRIGHT 2004 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2003-15594 BIOTECHDS
TITLE: Concurrent interrogation of a number of polymorphic sites, useful for genetic testing, carrier screening, genetic profiling, and identity testing, comprises conducting a multiplexed elongation assay using probes; for use in polymorphism detection and expression profiling
AUTHOR: LI A X; HASHMI G; SEUL M
PATENT ASSIGNEE: BIOARRAY SOLUTIONS LTD
PATENT INFO: WO 2003034029 24 Apr 2003
APPLICATION INFO: WO 2002-US33012 15 Oct 2002
PRIORITY INFO: US 2002-364416 14 Mar 2002; US 2001-329427 15 Oct 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-393553 [37]
AN 2003-15594 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - Concurrent interrogation of a number of polymorphic sites, comprises conducting a multiplexed elongation assay by: (a) applying one or more temperature cycles to achieve linear amplification of the target; or (b) a combination of annealing and elongation steps under temperature-controlled conditions.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) a method of concurrent determination of nucleotide composition at designated polymorphic sites located within one or more target nucleotide sequences; (2) methods of sequence-specific amplification of assay signals produced in the analysis of a nucleic acid sequence of interest in a biological sample; (3) a method of forming a covering probe set for the concurrent interrogation of a designated polymorphic site located in one or more target nucleic acid sequences; (4) methods of identifying or determining polymorphisms at one or more, or two or more, designated sites of a target nucleotide sequence or within a target polynucleotide sequence; (5) a method of concurrent interrogation of a nucleotide composition at S polymorphic sites, P sub S = (c sub P (s); 1 at most s at most S) located within one or more contiguous target sequences; (6) a method of concurrently determining a configuration of multiple designated sites in one or more targets; (7) a method of determining a configuration of at least one designated site in one or more nucleic acids; (8) a method for the concurrent interrogation of a set of designated polymorphisms within one or more nucleic acids; (9) methods of determining a sequence of a nucleic acid; (10) methods of determining a sequence of at least one designated site in one or more nucleic acids; (11) a **kit** for determining the sequence of designated polymorphic sites of a nucleic acid comprising several probes, where each probe contains a terminal elongation initiation region that is designed to align with a portion of the nucleic acid at the designated site and that is capable of initiating an elongation of the probe, and a variable duplex anchoring region designed to align with different portions of the

nucleic acid; (12) a method of amplifying a nucleic acid; (13) a method of determining the composition of polymorphic sites in a nucleic acid; (14) methods of detecting an elongation reaction; and (15) probes selected from the group of probes listed in the specification.

BIOTECHNOLOGY - Preferred **Kit**: The probes are formed into an encoded array or probes. The **kit** further comprises means for performing an assay to anneal the probes to the nucleic acid, and the assay further comprises a means for elongating the annealed probes. The **kit** also comprises means for detecting which probes are elongated. Preferred Method: Concurrent determination of nucleotide composition at designated polymorphic sites located within one or more target nucleotide sequences comprises: (a) providing one or more sets of probes, each probe capable of annealing to a subsequence of target nucleotide sequence(s) located within a range of proximity to a designated polymorphic site; (b) contacting the set of probes with the target nucleotide sequences to permit formation of hybridization complexes by placing an interrogation site within a probe sequence in direct alignment with the designated polymorphic site; (c) determining the presence of a match or a mismatch between the interrogation site and a designated polymorphic site, for each hybridization complex; and (d) determining the composition of the designated polymorphic site. The target nucleotide sequences are produced in a multiplex PCR reaction using one or more **primer** sets. The **primer** sets are degenerate **primer** sets. The targets are fragments of genomic DNA or of cDNA. The probes are spatially encoded on a substrate, or immobilized on encoded microparticles. The encoded microparticles are assembled into a random encoded array. Each probe contains a terminal elongation initiation region capable of initiating an elongation or extension reaction. The reaction is catalyzed by a polymerase lacking 3'-5' exonuclease activity. Determining the presence of a match or a mismatch comprises adding one or more deoxynucleotide triphosphates, where the method further comprises using a polymerase capable of extending or elongating probes. At least one of the deoxynucleotide triphosphates is labeled to generate an optically detectable signature associated with the elongation product. An optical label is attached to one or more probes by annealing to the probes a fluorescently labeled target to form a fluorescent hybridization complex. The method further comprises using a polymerase capable of extending or elongating probes displaying a match by addition of one or more deoxynucleotide triphosphates to form an elongated hybridization complex, and identifying elongation products by detecting the stability of optical signatures under conditions in which temperature is set to a value above the melting temperature of any hybridization complex formed by target and non-matched probe but below the melting temperature of any extended hybridization complex formed by target and elongated probe. One or more probes from the set of probes are immobilized on encoded microparticles and a change in optical signature is detected. The arrays are arranged in a spatially encoded manner. The change in optical signature is detected and particle identity is determined. Sequence-specific amplification of assay signals produced in the analysis of a nucleic acid sequence of interest in a biological sample comprises: (a) providing a set of immobilized probes capable of forming a hybridization complex with the sequence of interest; (b) contacting the set of immobilized probes with the biological sample containing the sequence of interest under conditions which permit the sequence of interest to anneal to at least one of the immobilized probes to form a hybridization complex; (c) contacting the hybridization complex with a polymerase to allow elongation or extension of the probes contained within the hybridization complex; (d) converting elongation or extension of the probes into an optical signal; and (e) recording the optical signal from the set of immobilized probes in real time. The method further comprises performing one or more cycles, each cycle comprising annealing-extending/elongating-detecting-denaturing steps, where each cycle results in the increase of the number of extended or elongated probes in arithmetic progression. The method comprises setting

a first temperature favoring the formation of a hybridization complex, setting a second temperature favorable to polymerase-catalyzed extension, converting extension or elongation into optical signal, recording/imaging optical signal/signatures from all probes, and setting a third temperature to ensure denaturation of all hybridization complexes. Sequence-specific amplification of assay signals produced in the analysis of a nucleic acid sequence of interest in a biological sample, where the method permits real-time monitoring of amplified signal, comprises: (a) providing a temperature-controlled sample containment device with associated temperature control apparatus permitting real-time recording of optical assay signal produced within the device; (b) providing within the sample containment device a set of distinguishable, immobilized oligonucleotide probe capable forming a hybridization complex with the sequence of interest; (c) permitting the sequence to anneal to the set of immobilized oligonucleotide probes to form a hybridization complex; (d) contacting the hybridization complex with a polymerase to allow elongation of extension of the matched probes contained within a hybridization complex; (e) providing means to convert elongation or extension of matching probes into an optical assay signal; (f) providing an optical recording/imaging device capable of recording optical assay signals from the set of immobilized probes in real time; (g) performing one or more annealing-extending-detecting-denaturing cycles, each cycle increasing the number of extended or elongated probes in arithmetic progression. Forming a covering probe set for the concurrent interrogation of a designated polymorphic site located in one or more target nucleic acid sequences comprises: (a) determining the sequence of an elongation probe capable of alignment of the interrogation site of the probe with a designated polymorphic site; (b) further determining a complete set of degenerate probes to accommodate all non-designated as well as non-selected designated polymorphic sites while maintaining alignment of the interrogation site of the probe with the designated polymorphic site; and (c) reducing the degree of degeneracy by removing all tolerated polymorphisms. The covering set contains at least two probes with different interrogation site composition per designated site. The reduction of complexity is accomplished by probe pooling. Identifying polymorphisms at one or more designated sites on one or more target nucleotides comprises: (a) providing one or more probes capable of interrogating the designated sites; (b) forming an elongation product by elongating one or more probes designed to interrogate a designated site; and (c) determining the compositions at two or more sites. The method further comprises forming a hybridization complex by annealing to the elongation product a second probe designed to interrogate a second designated site. Identifying polymorphisms at one or more designated sites within a target polynucleotide sequence comprises providing one or more probes capable of interrogating the designated sites, assigning a value to each designated site while accommodating non-designated polymorphic sites located within a range of proximity to each polymorphism. The homology between the probes and the target sequence is analyzed by multiplexing. Determining polymorphisms at one or more designated sites of a target nucleotide sequence comprises providing one or more pairs of probes capable of detecting deletions, where the deletions are placed at the 3' terminus of the probe or within 3-5 bases of the 3' terminus. Alternatively, the method comprises providing a probe set for the designated sites and grouping the probe set in different probe subsets according to the terminal elongation initiation of each probe. The method further comprises multiplexing the probe set, measuring each probe in the probe set without interference from the other probes in the probe set and changing the allele matching pattern of a target polynucleotide sequence to include alleles that are tolerated by a probe set. Changing the allele matching pattern of a target polynucleotide sequence comprises pooling one or more probe sets to include matched allele, or comparing the signal intensities produced by the probe set. The method further comprises separating the terminal elongation initiation region and duplex anchoring region on the probe set.

Identifying polymorphisms at two or more designated sites of a target nucleotide sequence comprises selecting a multiplicity of designated polymorphic sites to permit allele assignment, providing two or more probes capable of concurrent interrogation of the multiplicity of designated sites, assigning a value to each designated site, and combining the values to determine the identity of an allele or group of alleles while accommodating non-designated sites near the designated polymorphisms. Concurrent interrogation of a nucleotide composition at S polymorphic sites, P sub S = (c sub P (s); 1 at most s at most S) located within one or more contiguous target sequences comprises assigning to each c sub P one of a limited set of possible values by performing the following steps: (a) providing a set of designated immobilized oligonucleotide probes, also known as elongation probes, each probe capable of annealing in a preferred alignment to a subsequence of the target located proximal to a designated polymorphic site, the preferred alignment placing an interrogation site within the probe sequence in direct juxtaposition to the designated polymorphic site, the probes further containing a terminal elongation initiation (TEI) region capable of initiating an elongation or extension reaction; (b) permitting the target sequences to anneal to the set of immobilized oligonucleotide probes to form probe-target hybridization complexes; and (c) for each probe-target hybridization complex, calling a match or a mismatch in composition between interrogation site and corresponding designated polymorphic site. The probes are immobilized in a spatially encoded fashion on a substrate, or on encoded microparticles, which are assembled in a random encoded array on a substrate. The calling step involves the use of a polymerase capable of extending or elongating probes whose interrogation site composition matches that of the designated polymorphic site in the target, and only those probes, by addition of one or more nucleoside triphosphates, one of which is labeled to generate an optically detectable signature. The probes are immobilized on encoded microparticles and the change in optical signature is detected, and particle identity determined, by flow cytometry or by direct imaging. Concurrently determining a configuration of multiple designated sites in one or more targets comprises: (a) providing **primers** for amplification of the target(s), providing a probe array, using the probe array to perform an assay that produces an elongation product; and (b) detecting the elongation product. Determining a configuration of at least one designated site in one or more nucleic acids comprises: (a) providing several copies of one or more nucleic acids, each nucleic acid having at least one polymorphism; (b) choosing at least one polymorphism as a designated site; (c) providing two or more types of probes capable of concurrently interrogating each designated site to determine a composition of each designated site; (d) contacting the probes to the copies of one or more nucleic acids under conditions that cause at least some probes to hybridize to the nucleic acids; (e) performing an elongation reaction that elongates members of the probe set that are annealed to the nucleic acid; (f) detecting the elongation reaction; and (g) assigning a value to each designated site. Each type of probe comprises a terminal elongation region that is designed to align with a portion of the nucleic acid at the designated site and that is capable of initiating a polymerase-catalyzed elongation of the probe, and a variable duplex anchoring region designed to align with different portions of the nucleic acid. The method further comprises combining the values to determine the identity of each designated site. The value assigned to the designated site corresponds to the nucleotide identity at the designated site. The value assigned to the designated site corresponds to a 1 if the probe perfectly matches the nucleic acid and a 0 if the probe does not perfectly match the nucleic acid. The values are combined to determine the identity of an allele or group of alleles. The terminal elongation initiation region is immediately adjacent to the duplex anchoring region, or is linked to the duplex anchoring region by a molecular tether. The duplex anchoring regions of the probes in the probe set are designed to take into account polymorphism in non-designated sites. The nucleic acids

are obtained from the cystic fibrosis conductance transmembrane regulator (CFTR) gene or the human leukocyte antigen (HLA) gene. The probes align with a target sequence of a sense or antisense DNA strand. Homology between the probes and the target sequence is analyzed in parallel reactions. At least one polymorphism is a deletion, an insertion, or a single nucleotide polymorphism. Each probe is designed to detect deletions, where the portion is at the 3' terminus or within 3-5 bases of the 3' terminus. Probes with the same terminal elongation region are grouped together. Concurrent interrogation of a set of designated polymorphisms within one or more nucleic acids comprises: (a) providing several copies of one or more nucleic acids, which contain a set of designated polymorphisms; (b) contacting the nucleic acids to several types of probes, where each type of probe is capable of annealing to the nucleic acid(s) and has a different length than the other types of probes; (c) annealing the nucleic acid(s) to the probes; (d) elongating the probes; (e) detecting the elongated probes; and (f) determining the sequence of the designated polymorphism in the nucleic acid. Determining a sequence of a nucleic acid comprises providing several copies of nucleic acids having at least one polymorphism, choosing at least one polymorphism as a designated site, providing several types of probes cited above, where the probes are coupled to one or more solid supports, and performing an assay to determine a sequence of the nucleic acid. Determining a sequence of at least one designated site in one or more nucleic acids comprises: (a) providing a buffer solution comprising several copies of one or more nucleic acids having polymorphic sites, with at least one polymorphic site chosen as a designated site, two or more types of probes, a polymerase, and several types of dNTPs, where at least one type of **dNTP** is labeled; (b) heating the buffer solution to a first temperature to cause annealing of the probes to the nucleic acid(s); (c) heating the buffer solution to a second temperature to cause elongation of the annealed probes; and (d) detecting the elongated probes to determine the sequence of the designated sites of the nucleic acids. Amplifying a nucleic acid comprises providing several types of probes that are coupled to several types of encoded beads, where each type of probe is coupled to only one type of encoded bead, providing a template molecule capable of annealing to at least one type of probe, performing at least one cycle of amplification, which comprises annealing the template to at least one type of probe, elongating the annealed probes, and denaturing annealed template. Determining the composition of polymorphic sites in a nucleic acid comprises: (a) providing a nucleic acid having polymorphic sites; (b) choosing at least two polymorphic sites as designated sites; (c) providing two or more probes capable of interrogating the designated sites; (d) interrogating the designated sites so that the presence of the designated sites is established and compositions at the sites are determined. Interrogating the designated sites comprises: (a) forming a hybridization complex between a first probe and the nucleic acid; (b) forming an elongation product by elongating the first probe; and (c) forming a hybridization complex by annealing to the elongation product a second probe designed to interrogate a second designated site. One or more hybridization steps at additional designated sites follow the hybridization step. Detecting an elongation reaction comprises: (a) providing several probes; (b) coupling the probes to encoded beads that permit the identification of the probes; (c) contacting the encoded beads with a solution containing nucleic acids and dATP, dCTP, dTTP, and dGTP, where one type of the dNTPs comprises a label, or with a solution containing one labeled **ddNTP** and three dNTPs; (d) providing a polymerase; (e) performing an elongation reaction; and (f) detecting a product of an elongation reaction. The label is a fluorescent tag. Alternatively, the method comprises providing several probes, coupling the probes to encoded beads that permit the identification of the probes, contacting the encoded beads with a solution containing dATP, dCTP, dTTP, and dGTP, providing a polymerase, performing an elongation reaction to obtain an elongated probe, providing a labeled oligonucleotide probe designed to be complementary to a portion

of the elongated probe, annealing the probe to the elongated probe, and detecting the elongated probe. The method may also comprise: (a) providing a labeled target sequence, providing several types of probes, where one type of probes is exactly complementary to the labeled target sequence; (b) coupling the probes to encoded beads that permit the identification of the types of probes; (c) annealing the labeled target to the probes; (d) performing an elongation reaction to obtain elongated probes; (e) heating annealed probes to a temperature, the temperature being sufficient to denature duplex structures containing probes that do not match while preserving duplex structures corresponding to perfectly matched probes; and (f) identifying the elongation product corresponding to perfectly matched probes by determining which encoded beads have a bound target sequence after heating.

USE - The probes and methods are useful for identifying or detecting polymorphisms at one or more designated sites. The methods are useful for identifying mutations within the cystic fibrosis conductance transmembrane regulator (CFTR) or the human leukocyte antigen (HLA) gene. Concurrent interrogation of a multiplicity of polymorphic sites is useful for genetic testing, carrier screening, genotyping or genetic profiling, and identity testing. The methods are also useful for improving the reliability and accuracy of polymorphism analysis of target regions containing polymorphic sites in addition to the polymorphic sites designated for interrogation.

EXAMPLE - Genomic DNA, extracted from several patients, was amplified with corresponding **primers** in a multiplexed polymerase chain reaction (PCR) (mPCR). Following amplification, products were purified to remove all reagents using a commercially available **kit**. DNA concentration was determined by spectrophotometric analysis. Single or pooled PCR products (20 ng each) were added to an annealing mixture containing 10 mM Tris-hydrochloric acid, 1 mM ethylene diamine tetraacetic acid (EDTA), 0.2 M sodium chloride, 0.1% Triton X-100. The annealing mixture was mixed with elongation mixture containing 3 U of thermo sequenase, 1 x enzyme buffer with fluorescein-labeled or TAMRA-labeled **dNTP** analogs and 1-10 micromole of each type of unlabeled **dNTP** and placed in contact with an array of oligonucleotide probes display on a color-coded array. The annealing and elongation reactions were allowed to proceed in a temperature-controlled cycler. The temperature steps were three minutes each at 65 degreesC, 60 degreesC, 55 degreesC, 50 degreesC and 45 degreesC, with a ramp between temperatures of less than 30 seconds. The bead array was washed with distilled water for 5-15 minutes and an image containing the fluorescence signal from each bead within the array was recorded using a fluorescence microscope equipped with a CCD camera. Images were analyzed to determine the identity of each of the elongated probes.(143 pages)

L7 ANSWER 5 OF 15 BIOTECHDS COPYRIGHT 2004 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2003-10619 BIOTECHDS
TITLE: Reducing the complexity of a nucleic acid sample by providing non-gel based methods for size fractionation, useful for the analysis of genomic DNA, genotyping individuals, diagnosis of disease and/or predisposition to disease;
size fractionation, DNA **primer**, DNA probe and DNA array useful for pharmacogenomics, forensics analysis, plant breeding and animal breeding
AUTHOR: SU X; MATSUZAKI H; KENNEDY G
PATENT ASSIGNEE: AFFYMETRIX INC
PATENT INFO: WO 2003010328 6 Feb 2003
APPLICATION INFO: WO 2002-US23570 23 Jul 2002
PRIORITY INFO: US 2001-916135 25 Jul 2001; US 2001-916135 25 Jul 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-229646 [22]
AN 2003-10619 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - Reducing the complexity of a first nucleic acid sample to produce a second nucleic acid sample, comprising fragmenting a first nucleic acid to produce fragments, ligating one or more adapters to the fragments, amplifying a plurality of the fragments by PCR, and modulating the size of the amplified fragments by varying one or more reaction conditions or reagents to reduce the complexity of the first nucleic acid sample.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) a method for analyzing a first nucleic acid sample, comprising obtaining a second nucleic acid sample by fragmenting the first nuclei acid sample to produce fragment, ligating one or more adaptors of the fragments, and amplifying the fragments by PCR, where fragments of a specific size range are preferentially amplified by varying one or more of the reaction conditions or reagents, such as extension time, annealing time, **primer** concentration, **primer** length, presence or absence of a 3' to 5' exonuclease activity, and concentration of nucleotide analogues, providing a nucleic acid array, hybridizing the second nucleic acid sample to the array, and analyzing a hybridization pattern resulting from the hybridization; (2) a method of screening for DNA sequence variations in an individual, comprising providing a first nucleic acid sample from the individual, obtaining a second nucleic acid sample by fragmenting the first nucleic acid sample to produce fragments, ligating adaptor sequences to the fragments, and amplifying a subset of the fragments by a PCR, where one or mere reaction conditions or reagents are varied to favor amplification of a subset of fragments of a specific size range, providing a nucleic acid array where the array comprises probes designed to interrogate for DNA sequence variations, hybridizing the second nucleic acid sample to the array, generating a hybridization pattern resulting from the hybridization, and determining the presence or absence of DNA sequence variations in the individual based upon an analysis of the hybridization pattern; (3) a method of screening for DNA sequence variations in a population of individuals, comprising providing a first nucleic acid sample from each of the individuals, obtaining a second nucleic acid sample by fragmenting the first nucleic acid sample to produce fragments, ligating adaptor sequences to the fragments, and amplifying a subset of the fragments by a PCR, where one or mere reaction conditions or reagents are varied to favor amplification of a subset of fragments of a specific size range, providing a plurality of nucleic acid arrays where the arrays comprise probes designed to interrogate for DNA sequence variations, hybridizing each of the second nucleic acid sample to the plurality of arrays, generating a plurality of hybridization patterns resulting from the hybridization, and determining the presence or absence of DNA sequence variations in a population of individuals based upon an analysis of the hybridization patterns; (4) a method of reducing the complexity of a first nucleic acid sample to produce a second nucleic acid sample where the second nucleic acid sample is obtainable by fragmenting the first nucleic acid sample to produce fragments, ligating adaptor sequences to both ends of the fragments such that the 5' and 3' ends of the fragments are complementary to one another, and amplifying a subset of the fragments by PCR where a subset of fragments of a specific size range are preferentially amplified by varying the PCR **primer** concentration; (5) a method of genotyping an individual, comprising identifying a collection of single nucleotide polymorphisms (SNPs) that are found on fragments of a selected size range resulting from digestion with one or more selected restriction enzymes, designing an array to interrogate the collection of SNPs, providing a first nucleic acid sample from the individual, fragmenting the first nucleic acid sample with the one or more selected restriction enzymes, amplifying the fragments by PCR where a subset of fragments of the selected size range are preferentially amplified, hybridizing the PCR product to an array, and analyzing the hybridization pattern to determine the presence or absence of the collection of SNPs; and (6) a **kit** for reducing the complexity of a nucleic acid sample, comprising a buffer, nucleotide triphosphates, a reverse transcriptase, a nuclease,

one or more restriction enzymes, one or more adaptors, a ligase, a DNA polymerase, one or more **primers** and instructions for the use of the **kit**.

BIOTECHNOLOGY - Preferred Method: The reaction condition or reagent varied in the method of reducing the complexity of a first nucleic acid sample to produce a second nucleic acid sample, is extension time, annealing time, **primer** concentration, **primer** length, presence or absence of 3' to 5' exonuclease activity, and concentration of nucleotide analogues. The adaptors are designed so that the 5' and 3' ends of the fragments are complementary to one another. The complementarity is at least 10 bases long and is within 50 or 100 bases of the end of the fragments. When the reaction condition varied is the extension time of the PCR. The extension time is 2-5, 5-10 or 10-30 seconds. When the reaction condition varied is **primer** concentration, where the **primer** concentration is 0.1- 1, 0.1-10, 0.5-2.0 or 0.3-0.5 microM. When the reaction condition varied is **primer** length, the **primer** length is 10 to 100, 15-50 or 20-35 bases. The reaction condition varied is also the presence or absence of a 3' to 5' exonuclease activity, or the inclusion of one or more strand terminating nucleotides that are dideoxyribonucleotide triphosphates, such as ddATP, ddTTP, ddGTP, ddCTP and ddtUTP. The ratio of **dNTP** to **ddNTP** is 100 to 1 or 1000 to 1. The method further comprises fractionating the fragments according to size by gel filtration chromatography prior to amplification. The step of fragmenting the first nucleic acid sample comprises digestion with at least one restriction enzyme, and digestion with a restriction enzyme that has a six base recognition sequence. The adaptor sequences comprise PCR **primer** template sequences. The second nucleic acid sample comprises at least 0.01%, 0.5%, 3%, 12% or 50% of said first nucleic acid sample. The first nucleic acid sample is genomic DNA, DNA, cDNA derived from RNA or mRNA. The size range of a substantial amount of the amplified DNA is 100-1000, 200-1200 or 400 or 800 base pairs. The method for analyzing a first nucleic acid sample comprises determining whether the first nucleic acid sample contains sequence variations, preferably SNPs. The nucleic acid array is designed to query DNA fragments which have been produced by the procedures used to obtain the second nucleic acid sample. A substantial amount of the sequences predicted to be contained in the second nucleic acid sample are predetermined. A substantial amount of the sequences predicted to be contained in the second nucleic acid sample are first determined by a computer system. The method for analyzing a first nucleic acid sample comprises determining whether the first nucleic acid sample contains sequence variations, preferably SNPs. The nucleic acid array is designed to query DNA fragments which have been produced by the procedures used to obtain the second nucleic acid sample. A substantial mount of the sequences predicted to be contained in the second nucleic acid sample are predetermined. A substantial amount of the sequences predicted to be contained in the second nucleic acid sample are first determined by a computer system. The sequence variation in the method of (2) or (3) is an SNP. The SNP in the method of (2) is associated with a disease or with the efficacy of a drug. The method of (3) further comprises diluting the product of the PCR and amplifying the diluted product by a second round of PCR. The **primer** concentration in the method of (4) is 0.1-1, 0.1-10, 0.5-2.0 or 0.3-0.5 microM.

USE - The methods and compositions of the present invention are useful for the analysis of genomic DNA, SNP discovery, genotyping individuals, diagnosis of disease and predisposition to disease, pharmacogenomics, determination of relatedness, forensics and marker assisted breeding of animals and plants.

EXAMPLE - 5 microliters of 0.1 micrograms/microliters human genomic DNA was digested in a 20 microliters reaction volume with 20 units restriction enzyme, 1 x RE (restriction enzyme) buffer and 1 micrograms/microliters BSA (bovine serum albumin) for 2 hours at 37 degreesC. The 20 microliters restriction digest was then mixed with 0.5 microliters of 25 microM adaptor, 2 microliters 100 mM DTT, 2.5

microliters 10 mM ATP and 0.25 microliters 2000 units/microliters ligase. For PCR (polymerase chain reaction) 2 microliters of the ligation reaction was amplified in a 100 microliters reaction with concentrations of **primer** varying from 0.4-0.8 microM, 250 microM dNTPs, 2 mM MgCl2, and 5 units TaqGold (RTM) polymerase. The product was concentrated to about 40 microliters using a filter unit or a Qiagen PCR clean up **kit**, then fragmented with DNase. The results of the hybridization of the PCR products to an array designed to detect the presence or absence of a given SNP containing target sample showed approximately 13000 SNPs. The first nucleic acid was digested with BglII and the desired range was 400-700 bp.(46 pages)

L7 ANSWER 6 OF 15 BIOTECHDS COPYRIGHT 2004 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2003-21282 BIOTECHDS
TITLE: Microorganism detecting composition comprises **dideoxynucleotide triphosphate**(s) corresponding to one of four **deoxynucleotide triphosphate**, and thermally stable polymerase enzyme; **dNTP** and DNA **primer** for DNA sequencing and microorganism detection
AUTHOR: LEUSHNER J; HUI M; DUNN J M; LACROIX J
PATENT ASSIGNEE: LEUSHNER J; HUI M; DUNN J M; LACROIX J
PATENT INFO: US 2003082535 1 May 2003
APPLICATION INFO: US 2001-802110 7 Mar 2001
PRIORITY INFO: US 2001-802110 7 Mar 2001; US 1996-640672 1 May 1996
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-576607 [54]
AN 2003-21282 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - Microorganism detecting composition comprises at least one **dideoxynucleotide triphosphate** which is in a mole ratio to a corresponding **deoxynucleotide triphosphate** of 1:50-1:500, and a thermally stable polymerase enzyme that incorporates dideoxynucleotides into an extending nucleic acid polymer at a rate which is not less than 0.4 times the rate of incorporation of deoxynucleotides.

DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for a **kit** for detection of a target microorganism, which comprises, in packaged combination, a pair of **primers** which bind to the sense and antisense strands, respectively, and flank a selected region within the genome target microorganism; and the inventive microorganism detecting composition.

BIOTECHNOLOGY - Preferred Composition: The mole ratio **dideoxynucleotide triphosphate** to **deoxynucleotide triphosphate** is 1:100-1:300. Preferred **Kit**: The mole ratio **dideoxynucleotide triphosphate** to its corresponding **deoxynucleotide triphosphate** is 1:100-1:1000, preferably 1:500. At least one of the **primers** is labeled with a fluorescent label. The **primers** are each labeled with a spectroscopically-distinct fluorescent label. The target microorganism is Chlamydia trachomatis, human immunodeficiency virus, or human papilloma virus. The first and second **primers** are oligonucleotides including TCCGGAGCGAGTTACGAAGA; ATTCAATGCCCGGGATTGGT; CCGACCGCGTCTTGAAAACAGATGT; CACCCACATTCCCAGAGAGCT; CGTGCAGCTTTGTGGGAATGT; CTAGATTTCATCTTGTTCAATTGC; AGCATGCGTRTKGGTTACTAYGG; TGACTTTGTTTTCGACCGYGTTTT; CTAAAGTYGCRCATCCACATTCC; CATCCACATTCCCASARAGCTGC; ATGCCCGGGATTGGTTGATC; GGAGACTTTGTTTTCGACCG; CATTCCCACAAAGCTGCGCG; TTCCCACAAAGCTGCGCGAG; CCCACAAAGCTGCGCGAGCG; ACCTTTCGGTTGAGGGAGAGTCTA; GGACCAATTCTTATTCCCAAGCGA; ATCACTCTTTGGCAACGACC; CAGGAGCAGATGATACAGTATTAG; GCMCAGGGWCATAAYAATGG; or CGTCCMAARGGAWACTGATC.

USE - The composition is used for detecting a target microorganism. It is used in a bi-directional DNA sequencing method in several contexts including detection of mutations, particularly mutations of medical

significance, in samples derived from a human patient, animal, plant, or microorganism; determination of HLA (human leukocyte antigen) type ancillary to transplant procedures; detection and identification of microorganisms, particularly pathogenic microorganisms, in a sample; and in-situ sequencing reactions to produce sequencing fragments within a histological specimen which are then removed from a selected location on the tissue preparation and loaded onto a gel for sequence analysis.

ADVANTAGE - The invention allows an evaluation to be directly performed on a natural abundance DNA sample. It provides for bi-directional sequencing of DNA which requires combining a complex DNA-containing sample with only a single reaction mixture, thus reducing risk of error and contamination, and increasing the ease with which the procedure can be automated.

EXAMPLE - Urine samples from patients suspected of carrying a sexually transmitted disease pathogen were prepared for sequence-based diagnosis. One hundred microlitres first void urine were deposited in a sterile microcentrifuge tube. One hundred microlitres Lysis Solution was added to the bacterial pellet and incubated 1 hour at 55 degrees Centigrade, or 18 hours at room temperature. After a final incubation at 95 degrees Centigrade for 10 minutes, 200 microlitres Geneclean II glass milk was added. DNA was eluted in 10 microlitres of double distilled water. The sample natural abundance DNA was treated with a pair of **primers**, i.e. TCCGGAGCGAGTTACGAAGA and ATTCAATGCCCGGGATTGGT, and reagents to identify the sequence of a C. trachomatis gene present in the sample. Three microlitres of the sequencing reaction mixture was placed in each of 4 tubes and covered with 1 drop mineral oil. The tube was heated for 3 minutes at 94 degrees Centigrade and cooled to 85 degrees Centigrade. Termination mixtures were added and the mixture was subjected to thermal cycling for 55 cycles. After the last cycle, the tubes were kept at 70 degrees Centigrade for 2 minutes, and cooled to 4 degrees Centigrade. Reaction products were electrophoretically separated and detected. The base-called sequence was compared to the known C. trachomatis sequence to confirm diagnosis, and results were reported to the patient file.(94 pages)

L7 ANSWER 7 OF 15 BIOTECHDS COPYRIGHT 2004 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2003-21512 BIOTECHDS
TITLE: Detecting a specific nucleotide variation at a defined site in a target nucleic acid polymer, useful for pre- or postnatal diagnosis of diseases, comprises extending the detection **primer** using labeled nucleotide triphosphates;
nucleotide variation detection for use in disease diagnosis and plant and animal breeding
AUTHOR: SODERLUND H E; SYVANEN A
PATENT ASSIGNEE: SODERLUND H E; SYVANEN A
PATENT INFO: US 2003082531 1 May 2003
APPLICATION INFO: US 1999-258216 26 Feb 1999
PRIORITY INFO: US 1999-258216 26 Feb 1999; US 1990-482005 16 Feb 1990
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-596956 [56]
AN 2003-21512 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - Detecting (M1) a specific nucleotide variation at a defined site in a target nucleic acid polymer, where a second nucleotide residue replaces the first nucleotide residue, comprises extending the **primer** using a polymerizing agent in a mixture containing one or more nucleoside triphosphates (NTPs), and detecting the incorporation of the NTP.

DETAILED DESCRIPTION - Detecting a specific nucleotide variation at a defined site in a target nucleic acid polymer, where a second nucleotide residue replaces the first nucleotide residue, comprises: (a) hybridizing a detectable amount of a target nucleic acid polymer in

single-stranded form with an oligonucleotide **primer**, where the detection **primer** comprises several nucleotide residues and is complementary to the nucleotide sequence of interest in a region disposed toward the 3' end from the defined site such that when the **primer** is hybridized to the polymer, there are no nucleotide residues between the defined site and the 3; end of the **primer** that are identical to the first or second nucleotide residues to be detected; (b) extending the **primer** using a polymerizing agent in a mixture containing one or more NTPs, and at least one NTP complementary to either the first or second nucleotide residue that comprises a means for detecting the incorporation of the NTP in a nucleic acid polymer, and optionally one or more chain terminating NTPs; and (c) detecting the incorporation of the NTP, where the identity of the nucleotide residue at the defined site is determined. INDEPENDENT CLAIMS are also included for the following: (1) detecting (M2) in a patient a predisposition to a genetic disorder resulting from a specific nucleotide variation at a defined site in a genetic material of the patient, where a first nucleotide residue is replaced by a second nucleotide residue, comprises obtaining a sample containing a detectable amount of genetic material obtained from the patient, and employing the steps of M1; (2) a **kit** for determining the specific nucleotide variations in a target nucleic acid polymer comprising in a packaged combination: (a) at least one amplification **primer** comprising an oligonucleotide complementary to and hybridizes with a portion of the target nucleic acid polymer and is effective as a **primer** for enzymatic nucleic acid polymerization and a first attachment moiety; (b) at least one detection step **primer** comprising an oligonucleotide complementary to and hybridizes with a portion 3' to a variable nucleotide of the target nucleic acid polymer; and optionally; (c) at least one solid support comprising a solid matrix and at least one attachment site which is capable of immobilizing the oligonucleotide of the amplification probe through the first attachment moiety; and (d) at least one NTP containing a means for detecting the incorporation of the NTP in a nucleic acid polymer; (3) a reagent for detecting the presence of a point mutation in which a normal nucleic acid residue is replaced by an abnormal nucleic acid residue at a defined site within gene of interest; (4) detecting at a defined site in the genome of a microorganism, the existence of point mutations leading to altered pathogenicity or resistance to therapy in microorganisms, where the first nucleotide residue is replaced by a second nucleotide residue, by obtaining a sample containing a detectable amount of genetic material obtained from the microorganism, and employing the steps of M1; and (5) detecting cells having a point mutation at a defined site in the genetic material, where the first nucleotide residue is replaced by a second nucleotide residue, and the mutated cells are mixed in a cell population with unmutated cells, by obtaining a detectable quantity of genetic material from the cell population while maintaining the ratio of mutated to unmutated cells, and employing the steps of M1. The cells are lymphocytes, where the mutated cells are leukemic cells.

BIOTECHNOLOGY - Preferred Method: M1 further comprise removing the extended detection step **primer** from the target nucleic acid polymer, and adding a second detection step **primer** complementary to the nucleotide sequence of interest in a region disposed toward the 3' end from the second defined site such that when the **primer** is hybridized to the immobilized polymer, there are no nucleotide residues between the second defined site and the 3' end of the **primer** that are identical to the third or fourth nucleotide residues to be detected. Detecting in a patient a predisposition to a genetic disorder resulting from a specific nucleotide variation at a defined site in a genetic material of the patient, where a first nucleotide residue is replaced by a second nucleotide residue, comprises obtaining a sample containing a detectable amount of genetic material obtained from the patient, and employing the steps of M1. M1 and M2 further comprise immobilizing the target nucleic acid polymer to a solid

support before hybridizing the target nucleic acid to an oligonucleotide polymer. The **primer** is complementary to a region of the nucleotide sequence of interest extending toward the 3' end of the target nucleic acid polymer from the nucleotide residue immediately adjacent to the defined site. The NTP comprising a means for detecting the incorporation of the NTP in a nucleic acid polymer is a deoxynucleoside triphosphate or a dideoxynucleoside triphosphate. The mixture includes a second NTP comprising a second means, different from the first, for detecting the incorporation of the second nucleotide triphosphate in a nucleic acid polymer. The extended product is eluted before determining the incorporation of the incorporated NTP. The nucleotide variations are detected in one single step by adding several detection **primers** and differently labeled NTPs identifying the variable nucleotide residues. The detectable amount of target nucleic acid polymer is obtained by performing a modified amplification reaction where at least one amplification **primer** comprises a first attachment moiety bonded to the **primer**. For the method of (4), the microorganism is human immunodeficiency virus, and the point of mutation is at a site selected from Asp67, Lys70 and Thr215. Preferred **Kit**: The **kit** for identifying nucleotide variation of apolipoprotein E polymorphism comprises a detection step **primer** having a sequence selected from: (p1) 5'-GCG CGG ACA TGG AGG ACG TG; (p2) 5'-ATG CCG ATG ACC TGC AGA AG; (p3) 5'-GTA CTG CAC CAG GCG GCC GC; and (p4) 5'-GGC CTG GTA CAC TGC CAG GC. The **kit** for detecting nucleotide variation in codon 6 of the human beta-globin gene causing sickle cell anemia comprises a detection step **primer** having the sequence: (p5) 5'-CAT GGT GCA CCT GAC TCC TG; or (p6) 5'-CAG TAA CGG CAG GCG GCC GC. The **kit** for detecting a nucleotide variation in codon 12 of the K-ras gene comprises a detection step **primer** having a sequence selected from: (p7) 5'-AAG GCA CTC TTG CCT ACG CCA; (p8) 5'-AGG CAC TCT TGC CTA CGC CAG; (p9) 5'-AAC TTG TGG TAG TTG GAG CT; and (p10) 5-ATC TGT GGT AGT TGG AGC TG. Preferred Reagent: The reagent comprises an oligonucleotide of sufficient length to act as a **primer** for an enzyme catalyzed chain extension nucleic acid polymerization reaction, where the oligonucleotide **primer** has a sequence which is complementary to a region of the gene of interest beginning with the nucleotide residue immediately adjacent to and toward the 3' end of the gene from the defined site and extending away from the defined site toward the 3' end of the gene, where an enzyme catalyzed chain extension nucleic acid polymerization will commence by adding a nucleic acid residue complementary to either the normal nucleic acid residue of the abnormal nucleic acid residue. The polynucleotide has a length of 10-40 nucleotide residues, and has a sequence selected from p1-p9.

USE - The method is useful for identifying specific point mutations and genetic variations. Specifically, the method can be used for pre- or postnatal diagnosis of hereditary predispositions or diseases, for the detection of somatic mutations in cancer, for the selection of cells and strains for industrial biotechnology and for plant and animal breeding.

ADVANTAGE - The new method provides several advantages over prior methods. The new method comprises few and easily performed procedures, thus especially suited for routine determinations of point mutations and nucleotide variations, allows the quantification of the proportion of mutated cells in a sample as well as the identification of mutations present in as little as 0.5% of the analyzed cell population, and is easily automated.

EXAMPLE - Four PCR **primers** (P1-P4) and 2 detection step oligonucleotide **primers** (D1 and D2) were synthesized. A biotinylated 5'-amino group was added to **primer** P2 with the aminolink II reagent. Leukocytic DNA was extracted from venous blood samples obtained from patients of known Apo E phenotype. DNA was amplified with P1 and P4 **primers**. An aliquot of the first PCR amplification mixture was transferred to a second PCR and directed by the biotinylated **primers** P2 and P3. The second amplification mixture was added with 5 microl of 5% suspension of avidin-coated

polystyrene particles, kept for 20degreesC for 30 minutes, collected by centrifugation, washed, and treated with NaOH. Suspension of particles was divided into 4 parts and collected by centrifugation in separate tubes. Particles carrying the DNA fragment were suspected in NaCl, MgCl2, Tris-HCL containing 2 pmol of the detection step **primer**. D1 oligonucleotide located immediately adjacent to the variable nucleotide 3745 was added to 2 of the tubes and D2 oligonucleotide adjacent to the variable nucleotide 3883 to 2 tubes. Oligonucleotide was annealed to the DNA template by heating the samples at 65degreesC for 2 minutes, and cooling them to 20degreesC. Dithiothreitol and (35S)-labeled deoxynucleoside triphosphates (**dNTP**) and dideoxynucleoside triphosphates (**ddNTP**) were added to yield 1 microM concentration of each final volume of 15 microl for identification of T: (35S)-dTTP, ddCTP and ddGTP to 2 tubes, one in which D1 and one in which D2 had been annealed; and (35S)-dCTP, ddTTP and ddGTP to 2 tubes, one in which D1 and one in which D2 had been annealed. T7 DNA polymerase was added to each tube and allowed the reaction to proceed. Products were eluted and radioactivity in liquid scintillation counter was measured. The differences in cpm values obtained in the T- and C-reactions allowed unequivocal identification of the variable nucleotide in both codons 112 and 118 in all 4 DNA samples. (16 pages)

L7 ANSWER 8 OF 15 BIOTECHDS COPYRIGHT 2004 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2003-25219 BIOTECHDS
TITLE: Detecting a specific nucleotide variation at a defined site in a target nucleic acid polymer, useful for pre- or postnatal diagnosis of diseases, comprises extending the detection **primer** using labeled nucleotide triphosphates;
DNA **primer** extension for use in disease diagnosis and plant and animal breeding
AUTHOR: SODERLUND H E; SYVANEN A
PATENT ASSIGNEE: SODERLUND H E; SYVANEN A
PATENT INFO: US 2003082530 1 May 2003
APPLICATION INFO: US 1995-465322 5 Jun 1995
PRIORITY INFO: US 1995-465322 5 Jun 1995; US 1990-482005 16 Feb 1990
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-708522 [67]
AN 2003-25219 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - Detecting (M1) a specific nucleotide variation at a defined site in a target nucleic acid polymer, where a second nucleotide residue replaces the first nucleotide residue, comprising extending the detection **primer** using a polymerizing agent in a mixture containing one or more nucleoside triphosphates (NTPs), and detecting the incorporation of the NTP, is new.

DETAILED DESCRIPTION - Detecting a specific nucleotide variation at a defined site in a target nucleic acid polymer, where a second nucleotide residue replaces the first nucleotide residue, comprises: (a) hybridizing a detectable amount of a target nucleic acid polymer in single-stranded form with an oligonucleotide **primer**, where the detection **primer** comprises several nucleotide residues and is complementary to the nucleotide sequence of interest in a region disposed toward the 3' end from the defined site such that when the **primer** is hybridized to the polymer, there are no nucleotide residues between the defined site and the 3' end of the **primer** that are identical to the first or second nucleotide residues to be detected; (b) extending the **primer** using a polymerizing agent in a mixture containing one or more NTPs, and at least one NTP complementary to either the first or second nucleotide residue that comprises a means for detecting the incorporation of the NTP in a nucleic acid polymer, and optionally one or more chain terminating NTPs; and (c) detecting the incorporation of the NTP, where the identity of the nucleotide residue at

the defined site is determined. INDEPENDENT CLAIMS are also included for: (1) detecting (M2) in a patient a predisposition to a genetic disorder resulting from a specific nucleotide variation at a defined site in a genetic material of the patient, where a first nucleotide residue is replaced by a second nucleotide residue, comprises obtaining a sample containing a detectable amount of genetic material obtained from the patient, and employing the steps of M1; (2) a **kit** for determining the specific nucleotide variation in a target nucleic acid polymer comprising in a packaged combination of: (a) at least one amplification **primer** comprising an oligonucleotide complementary to and hybridizes with a portion of the target nucleic acid polymer and is effective as a **primer** for enzymatic nucleic acid polymerization and a first attachment moiety; (b) at least one detection step **primer** comprising an oligonucleotide complementary to and hybridizes with a portion 3' to a variable nucleotide of the target nucleic acid polymer; and optionally; (c) at least one solid support comprising a solid matrix and at least one attachment site which is capable of immobilizing the oligonucleotide of the amplification probe through the first attachment moiety; and (d) at least one NTP containing a means for detecting the incorporation of the NTP in a nucleic acid polymer; (3) a reagent for detecting the presence of a point mutation in which a normal nucleic acid residue is replaced by an abnormal nucleic acid residue at a defined site within gene of interest; (4) detecting at a defined site in the genome of a microorganism, the existence of point mutations leading to altered pathogenicity or resistance to therapy in microorganisms, where the first nucleotide residue is replaced by a second nucleotide residue, by obtaining a sample containing a detectable amount of genetic material obtained from the microorganism, and employing the steps of M1; and (5) detecting cells having a point mutation at a defined site in the genetic material, where the first nucleotide residue is replaced by a second nucleotide residue, and the mutated cells are mixed in a cell population with unmutated cells, by obtaining a detectable quantity of genetic material from the cell population while maintaining the ratio of mutated to unmutated cells, and employing the steps of M1. The cells are lymphocytes, where the mutated cells are leukemic cells.

BIOTECHNOLOGY - Preferred Method: M1 further comprise removing the extended detection step **primer** from the target nucleic acid polymer, and adding a second detection step **primer** complementary to the nucleotide sequence of interest in a region disposed toward the 3' end from the second defined site such that when the **primer** is hybridized to the immobilized polymer, there are no nucleotide residues between the second defined site and the 3' end of the **primer** that are identical to the third or fourth nucleotide residues to be detected. Detecting in a patient a predisposition to a genetic disorder resulting from a specific nucleotide variation at a defined site in a genetic material of the patient, where a first nucleotide residue is replaced by a second nucleotide residue, comprises obtaining a sample containing a detectable amount of genetic material obtained from the patient, and employing the steps of M1. M1 and M2 further comprise immobilizing the target nucleic acid polymer to a solid support before hybridizing the target nucleic acid to an oligonucleotide polymer. The **primer** is complementary to a region of the nucleotide sequence of interest extending toward the 3' end of the target nucleic acid polymer from the nucleotide residue immediately adjacent to the defined site. The NTP comprising a means for detecting the incorporation of the NTP in a nucleic acid polymer is a deoxynucleoside triphosphate or a dideoxynucleoside triphosphate. The mixture includes a second NTP comprising a second means, different from the first, for detecting the incorporation of the second nucleotide triphosphate in a nucleic acid polymer. The extended product is eluted before determining the incorporation of the incorporated NTP. The nucleotide variations are detected in one single step by adding several detection **primers** and differently labeled NTPs identifying the variable nucleotide

residues. The detectable amount of target nucleic acid polymer is obtained by performing a modified amplification reaction where at least one amplification **primer** comprises a first attachment moiety bonded to the **primer**. For the method of (4), the microorganism is human immunodeficiency virus, and the point of mutation is at a site selected from Asp67, Lys70 and Thr215. Preferred **Kit**: The **kit** for identifying nucleotide variation of apolipoprotein E polymorphism comprises a detection step **primer** having a sequence selected from: (p1) 5'-GCG CGG ACA TGG AGG ACG TG; (p2) 5'-ATG CCG ATG ACC TGC AGA AG; (p3) 5'-GTA CTG CAC CAG GCG GCC GC; and (p4) 5'-GGC CTG GTA CAC TGC CAG GC. The **kit** for detecting nucleotide variation in codon 6 of the human beta-globin gene causing sickle cell anemia comprises a detection step **primer** having the sequence: (p5) 5'-CAT GGT GCA CCT GAC TCC TG; or (p6) 5'-CAG TAA CGG CAG GCG GCC GC. The **kit** for detecting a nucleotide variation in codon 12 of the K-ras gene comprises a detection step **primer** having a sequence selected from: (p7) 5'-AAG GCA CTC TTG CCT ACG CCA; (p8) 5'-AGG CAC TCT TGC CTA CGC CAG; (p9) 5'-AAC TTG TGG TAG TTG GAG CT; and (p10) 5-ATC TGT GGT AGT TGG AGC TG. Preferred Reagent: The reagent comprises an oligonucleotide of sufficient length to act as a **primer** for an enzyme catalyzed chain extension nucleic acid polymerization reaction, where the oligonucleotide **primer** has a sequence which is complementary to a region of the gene of interest beginning with the nucleotide residue immediately adjacent to and toward the 3' end of the gene from the defined site and extending away from the defined site toward the 3' end of the gene, where an enzyme catalyzed chain extension nucleic acid polymerization will commence by adding a nucleic acid residue complementary to either the normal nucleic acid residue of the abnormal nucleic acid residue. The polynucleotide has a length of 10-40 nucleotide residues, and has a sequence selected from p1-p10.

USE - M1 is useful for identifying specific point mutations and genetic variations. Specifically, the method can be used for pre- or postnatal diagnosis of hereditary predispositions or diseases, for the detection of somatic mutations in cancer, for the selection of cells and strains for industrial biotechnology and for plant and animal breeding.

ADVANTAGE - The new method provides several advantages over prior methods. The new method comprises few and easily performed procedures, thus especially suited for routine determinations of point mutations and nucleotide variations, allows the quantification of the proportion of mutated cells in a sample as well as the identification of mutations present in as little as 0.5% of the analyzed cell population, and is easily automated.

EXAMPLE - Four PCR **primers** (P1-P4) and 2 detection step oligonucleotide **primers** (D1 and D2) were synthesized. A biotinylated 5'-aminogroup was added to **primer** P2 with the aminolink II reagent. Leukocytic DNA was extracted from venous blood samples obtained from patients of known Apo E phenotype. DNA was amplified with P1 and P4 **primers**. An aliquot of the first PCR amplification mixture was transferred to a second PCR and directed by the biotinylated **primers** P2 and P3. The second amplification mixture was added with 5 mul of 5% suspension of avidin-coated polystyrene particles, kept for 20degreesC for 30 minutes, collected by centrifugation, washed, and treated with NaOH. Suspension of particles was divided into 4 parts and collected by centrifugation in separate tubes. Particles carrying the DNA fragment were suspected in NaCl, MgCl2, Tris-HCL containing 2 pmol of the detection step **primer**. D1 oligonucleotide located immediately adjacent to the variable nucleotide 3745 was added to 2 of the tubes and D2 oligonucleotide adjacent to the variable nucleotide 3883 to 2 tubes. Oligonucleotide was annealed to the DNA template by heating the samples at 65degreesC for 2 minutes, and cooling them to 20degreesC. Dithiothreitol and (35S)-labeled deoxynucleoside triphosphates (**dNTP**) and dideoxynucleoside triphosphates (**ddNTP**) were added to yield 1 muM concentration of each final volume of 15 mul for identification of T: (35S)-dTTP, ddCTP

and ddGTP to 2 tubes, one in which D1 and one in which D2 had been annealed; and (35S)-dCTP, ddTTP and ddGTP to 2 tubes, one in which D1 and one in which D2 had been annealed. T7 DNA polymerase was added to each tube and allowed the reaction to proceed. Products were eluted and radioactivity in liquid scintillation counter was measured. The differences in cpm values obtained in the T- and C-reactions allowed unequivocal identification of the variable nucleotide in both codons 112 and 118 in all 4 DNA samples. (16 pages)

L7 ANSWER 9 OF 15 BIOTECHDS COPYRIGHT 2004 THE THOMSON CORP. on STN
DUPLICATE 1
ACCESSION NUMBER: 2003-04214 BIOTECHDS
TITLE: A **primer** combination comprising at least one forward and reverse Human Immunodeficiency Virus (HIV)-1 **primer**, useful for genotyping of HIV-1 in samples, particularly those which have failed previous genotypic analysis;
DNA **primer** and reverse transcription-polymerase chain reaction for HIV virus infection diagnosis
AUTHOR: LLOYD R M; UZGIRIS A; HUONG J T
PATENT ASSIGNEE: VISIBLE GENETICS INC
PATENT INFO: WO 2002070731 12 Sep 2002
APPLICATION INFO: WO 2002-US6632 5 Mar 2002
PRIORITY INFO: US 2001-273683 5 Mar 2001; US 2001-273683 5 Mar 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-713456 [77]
AN 2003-04214 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - A **primer** combination (P) comprising (in a single solution) at least 1 forward Human Immunodeficiency Virus (HIV)-1 **primer** selected from among **primers** comprising a degenerate sequence of a fully defined sequence of 23 base pairs (bp), given in the specification, and at least 1 reverse HIV-1 **primer** comprising a degenerate sequence of a fully defined sequence of 30 or 31 bp, given in the specification, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) a genotyping **kit** comprising at least one forward HIV-1 **primer** from among **primers** comprising a degenerate sequence of a fully defined sequence of 23 bp, given in the specification, and at least one reverse HIV-1 **primer** comprising a degenerate sequence of a fully defined sequence of 30 or 31 bp, given in the specification; and (2) a method for evaluating a sample suspected of containing a non-B Group M HIV-1 virus or a Group O HIV-1 virus to assess the type of the virus comprising: (a) treating the sample to recover viral RNA; (b) reverse transcribing the recovered viral RNA; (c) sequencing the reverse transcription product; and (d) using the results of the sequencing step to establish the genotype of the tested virus (at least 1 of the reverse transcription step and the sequencing step is performed using the **primer** combination (P)).

BIOTECHNOLOGY - Preferred **Primer**: At least one forward **primer** comprises any of 8 fully defined sequences of 23-25 bp, given in the specification. At least one reverse **primer** comprises any of 7 fully defined sequences of 30-31 bp, given in the specification. The forward and reverse **primers** are members of a set of degenerate forward and reverse **primers**, and the **primer** combination includes at least two species of degenerate forward and at least two species of degenerate reverse **primers**. The forward and reverse **primers** comprise fully defined sequences of 24 and 30 bp, given in the specification, respectively. The forward **primers** have any of the 2 fully defined sequences of 23 bp, while the reverse **primers** have any of the 2 fully defined sequences of 30 bp, all given in the specification. The forward or reverse **primers** are labeled with a detectable label, where the

detectable label is a fluorescent label. Preferred **Kit**: At least one forward **primer** in the **kit** comprises any of 8 fully defined sequences of 23-25 bp, given in the specification. At least one reverse **primer** comprises any of 7 fully defined sequences of 30-31 bp, given in the specification. The forward and reverse **primers** are members of a set of degenerate forward and reverse **primers**, and the **primer** combination includes at least two species of degenerate forward and at least two species of degenerate reverse **primers**. The forward **primers** are members of the set of degenerate **primers** with any a fully defined sequence of 24 bp, given in the specification, where the **primers** have a any of 2 fully defined sequences of 23 bp, given in the specification. The reverse **primers** are members of the set of degenerate **primers** with any of fully defined sequence of 23 bp, given in the specification, where the **primers** have any of 2 fully defined sequences of 30 bp, given in the specification. The forward or reverse **primers** are labeled with a detectable label, where the detectable label is a fluorescent label. The **kit** further comprises one or more reagents selected from an RNase inhibitor, a reverse transcriptase, a polymerase, and **dNTP** and **ddNTP** feedstocks. Preferred Method: The method further comprises performing parallel genotyping procedures that is designed to evaluate B-subtype virus. The sample is one that has previously been the subject of a failed genotyping attempt using genotyping procedures that are designed to evaluate B-subtype virus.

USE - The compositions and methods of the present invention are useful for genotyping of HIV-1 containing samples, particularly those which have failed a previous genotyping analysis.

EXAMPLE - The TRUGENE (RTM) Human Immunodeficiency Virus (HIV)-1 genotyping **kit** was used to determine the genotype of certain non-B subtypes of HIV-1 virus. RNA was extracted from patient plasma samples. 17 microliters of RNA was added to the amplification master mix containing 0.2 microliters of a fully defined sequence of 24 bp, given in the specification, 0.4 microliters of a fully defined sequence of 30 bp, 6.4 microliters of water, 1.75 microliters of **dNTP**, 1.165 microliters of DTT and 0.58 microliters of RNase inhibitor. The reactions were thermocycled using a Perkin-Elmer 9700 thermocycler. After incubating the amplification master mix and the RNA together for 5 minutes at 50 degrees Centigrade, 14 microliters of a second master mix was added and the reverse transcriptase polymerase chain reaction (RT-PCR) cycle program continued. After the PCR cycle program was completed, samples were sequenced according to the protocol in the TRUGENE (RTM) HIV-1 package insert. (27 pages)

L7 ANSWER 10 OF 15 BIOTECHDS COPYRIGHT 2004 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2003-08245 BIOTECHDS
TITLE: Producing hybrid single-stranded DNA for genomic analysis, comprises producing outer and inner amplicons by nested polymerase chain reaction using **primers** that hybridize to the DNA, then forming ligatable and sequencing structures;
for use in genomics
AUTHOR: CHEN X
PATENT ASSIGNEE: UNIV VIRGINIA COMMONWEALTH
PATENT INFO: WO 2002090505 14 Nov 2002
APPLICATION INFO: WO 2002-US14431 9 May 2002
PRIORITY INFO: US 2001-289514 9 May 2001; US 2001-289514 9 May 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-111964 [10]
AN 2003-08245 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - Producing hybrid DNA with a single strand overhang that includes a target site, comprising obtaining a first, second and third

primer which hybridizes to a strand of a DNA, producing by nested polymerase chain reaction (PCR) using the **primers** as an outer and an inner amplicon, and forming a ligatable or a sequencing structure, is new.

DETAILED DESCRIPTION - Producing hybrid DNA with a single strand overhang that includes a target site, comprising: (a) obtaining a first **primer** which hybridizes to a 5' strand of a strand of DNA, and second and third **primers** that hybridize to a 3' strand of the strand of DNA; (b) producing by nested PCR using the first, second and third **primers**, an outer amplicon which includes the target site and an inner amplicon which excludes the target site; and (c) forming at least one of: (i) a ligatable structure which includes a 3'-5' sequence excluding the target site hybridized to a 5'-3' sequence which includes the target site; and (ii) a sequencible structure which includes a 5'-3' sequence excluding the target site hybridized to a 3'-5' sequence which includes the target site. INDEPENDENT CLAIMS are also included for the following: (1) genotyping DNA of an individual by analyzing at least one target site in the DNA , comprising employing the steps of the novel method, and analyzing the sequencing product formed by sequencing the sequencible structure, or the ligation product formed by ligating the ligatable structure with a labeled oligonucleotide, with a DNA sequencer to determine the genotype of the individual; (2) analyzing at least one target site in a DNA molecule, comprising:(a) amplifying by nested PCR the target site, where the PCR is carried out using inner and outer PCR **primer** pairs; (b) denaturing the first and second PCR products to form ssDNA sequences; (c) reannealing the ssDNA sequences to form sequencible and ligatable hybrid DNA molecules; (d) performing sequencing reactions with the sequencible hybrid DNA molecule and ligation reactions with the ligatable hybrid DNA molecule; and (e) determining the characteristics of the target site by analyzing results obtained in the performing step; (3) inner and outer PCR **primer** pairs for amplifying a target site in a DNA, where the outer PCR **primer** pair forms a first PCR product containing the target site, and where the inner PCR **primer** pair forms a second PCR product containing a portion of the first PCR product but does not contain the target site; (4) a **kit** for amplifying at least one target site in a DNA molecule, comprising the inner and outer PCR **primer** pairs of (3); and (5) a dideoxy DNA sequencing **kit** for producing short chain termination fragments, comprising dNTPs and ddNTPs present in a **dNTP:ddNTP** ratio ranging from 1:0-1:10.

BIOTECHNOLOGY - Preferred Method: In the methods of producing hybrid DNA with a single strand overhang that includes a target site, and in genotyping DNA of an individual by analyzing at least one target site in the DNA, the forming step forms both the ligatable structure and the sequencible structure. The target site includes at least one single nucleotide polymorphism. In genotyping the DNA of an individual, the sequencible structure is sequenced by a technique selected from dideoxy sequencing, pyrosequencing, and single base extension. The target sites are simultaneously analyzed by producing an ordered series of sequencing products of varying, non-overlapping length each specific for a particular target site, and the step of analyzing is carried out by electrophoresing the ordered series of sequencing products in a single channel of the DNA sequencer. Producing an ordered series of sequencing products is carried out by single base extension, or by dideoxy sequencing reaction using a low ratio of dNTPs to ddNTPs. The labeled oligonucleotide is fluorescently labeled. In analyzing at least one target site in a DNA molecule, the inner and outer **primer** pairs comprise a sequence tag that has a restriction enzyme recognition site. The step of amplifying is carried out using a low concentration of **primers**, and further comprises a second step of amplification which uses secondary **primers** for amplifying the sequence tags. In addition, the step is carried out in a single multiplex PCR reaction or in a multiple independent PCR reactions. The step analyzes one or more target sites. The results obtained in the performing step are analyzed by

the DNA sequencer.

USE - The method is useful for single nucleotide polymorphism genotyping based on a nested PCR design that creates structures directly suitable for both DNA sequencing and ligation reactions. The method improves our understanding of the genetics of complex traits, common diseases and drug response, and for individualized medicine.(46 pages)

L7 ANSWER 11 OF 15 BIOTECHDS COPYRIGHT 2004 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2003-01243 BIOTECHDS
TITLE: New aptamer comprising one base capable of base pairing and different from the standard Watson-Crick base, useful for isolating a specific ligand from a pool of ligands; DNA chip, DNA-polymerase, RNA-polymerase and reverse-transcriptase for DNA, RNA or peptide nucleic acid aptamer construction
AUTHOR: HAYASHIZAKI Y
PATENT ASSIGNEE: RIKEN KK; HAYASHIZAKI Y
PATENT INFO: WO 2002044195 6 Jun 2002
APPLICATION INFO: WO 2001-JP10400 28 Nov 2001
PRIORITY INFO: US 2000-253097 28 Nov 2000; US 2000-253097 28 Nov 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-608230 [65]
AN 2003-01243 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - An isolated aptamer (I) comprising at least one base capable of base pairing and different from the standard Watson-Crick (W-C) bases, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) preparing (M1) nucleic acid aptamers comprising at least one base capable of base pairing and different from the standard Watson-Crick (W-C) bases, involves providing a specific ligand, synthesizing a pool of nucleic acid aptamers comprising at least one base capable of base pairing and different from the standard W-C bases, mixing the pool of aptamers with the specific ligand, and selecting and amplifying a specific aptamer that binds to the specific ligand; (2) an insoluble substrate (II) having at least one (I) fixed to it; (3) a diagnostic **kit** (III) for the determination of a specific ligand selected from a pool of ligands comprising at least one (I) or (II);(4) a pharmaceutical composition (IV) comprising (I); (5) determining (M2) the nucleated base sequence of a nucleic acid template comprising at least one base capable of base pairing and different from the standard W-C bases, involves providing a nucleic acid template comprising at least one base capable of base pairing and different from the standard W-C bases, elongating the template using a **primer** or a promoter or a promoter and an initiator in the presence of a nucleic acid synthesizing enzyme, nucleic acid synthesizing enzyme substrates and nucleic acid enzyme substrate derivatives, and determining the base sequence of the templates as the reverse complement of the sequence of the elongation product obtained; (6) determining (M3) the nucleated base sequence of a nucleic acid template comprises at least one base capable of base pairing and different from the standard (W-C) bases, involves providing a nucleic acid template comprising at least one base capable of base pairing and different from the standard W-C bases, labeling one end of the template, chemically degrading the labeled template, and determining the length of the products obtained, obtaining the sequence of the template as the sequence of the incremental lengths of the products; (7) determining (M4) the nucleated base sequence of a nucleic acid template comprises at least one base capable of base pairing and different from the standard (W-C) bases, involves providing a nucleic acid template comprising at least one base capable of base pairing and different from the standard W-C bases, elongating the template using a **primer** or a promoter or a promoter and an initiator in the presence of a nucleic acid synthesizing enzyme, nucleic acid synthesizing enzyme substrates and nucleic acid

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		DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI; PLUR=YES; OP=ADJ	
☐	L1	((reaction vessel or vessel or tube or single tube or chamber) same primer)	15661
☐	L2	L1 same (dNTP or deoxynucleotide triphosphate)	2337
☐	L3	L2 same (ddNTP or dideoxynucleotide triphosphate)	113
☐	L4	L3 same (thermostable polymerase or thermal$ stable polymerase or Taq)	9
☐	L5	L4 same kit	3
☐	L6	L4 and kit	8
☐	L7	l2 and (thermostable polymerase or thermal$ stable polymerase or Taq)	1787
☐	L8	l2 same(thermostable polymerase or thermal$ stable polymerase or Taq)	1000
☐	L9	L8 same kit	129
☐	L10	L9 same (ddNTP or dideoxynucleotide triphosphate)	3
☐	L11	L9 and (ddNTP or dideoxynucleotide triphosphate)	10
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3. Product Info - StrataScript™ High Fidelity **RT**-PCR **Kit**
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5. Product Info - QuikChange® Multi Site-Directed Mutagenesis **Kit**
6. Product Info - StrataScript® One-Tube **RT**-PCR **kit** with Easy-A™ PCR Cloning Enzyme
7. Product Info - ProSTAR® HF **single**-tube **RT**-PCR System
8. Product Info - VariFlex™ Bacterial Expression Systems
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